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# THE CONTROL OF pH BY BUFFERS IN FISH TRANSPORT[1,2]

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## INTRODUCTION

The heavy fishing pressure on our Nation's streams and lakes which has developed in recent years has required large-scale propagation and planting of fishes, particularly trout, and has led to the development of the complex modern fish tanker.

One of the major problems confronting the fisheries biologist has been the development of better means for transporting fishes. Confinement of fishes in transport apparatus can produce critical conditions in the water which necessitate control if mortality is to be avoided. Several of these conditions have been investigated. However, one of these factors, that of increasing acidity, resulting from waste products and respiration, has not been adequately studied. We feel that an understanding of increasing acidity is not only of theoretical importance but also of great practical value. Such information should lead to more effective transport by serving as a basis for the development of better means for controlling water quality.

In this investigation several chemicals, capable of stabilizing acidity levels, have been studied. One of these, Tris-hydroxymethyl-aminomethane, has promise both for marine and fresh water application (this compound will henceforth be referred to as "tris-buffer"). This chemical can increase the acid-absorbing capacity of sea water by as much

[1] Submitted for publication May, 1958.
[2] Contribution Number 3, Marineland of the Pacific Biological Laboratory.

as 50 times without deleterious effects upon fishes. The application of this buffer has been studied in the laboratory and on several occasions successfully used during transport operations.

This study has been separated into two divisions: first, the practical aspects of buffer application to fish transport, and second, the theoretical and experimental background underlying the practical methods.

## ACKNOWLEDGMENTS

We wish to thank Dr. Boyd W. Walker of the University of California, Los Angeles, for his suggestions during the course of this research and for his helpful review of the manuscript. We also thank the staffs of the Steinhart Aquarium, the Waikiki Aquarium and members of the Territory of Hawaii, Division of Fish and Game, for their assistance during the transport operations reported here. We wish to thank Muriel Johnson for her help during preparation of the manuscript.

## THE PRACTICAL ASPECTS OF BUFFER APPLICATION TO FISH TRANSPORT

Transport operations which involve heavy loads, long distances, or poor water supply are apt to be limited by increasingly acid waters which may result in debilitation or death of fish. Under these circumstances it is desirable to stabilize pH in some way. Tris-buffer has proved to be the most effective for this purpose of several compounds tested. It was nontoxic to all fish species examined.

### Application of Tris-buffer

Tris-buffer is a highly stable, soluble white crystalline compound.[3] It is applied by dissolving in the transport water. Tests indicate that fishes can stand high concentrations (to 20 grams per gallon) of buffer with no ill effects. Light dosages (two to five grams per gallon) will generally be adequate to stabilize pH during transport of fish for moderate distances and while carrying moderate weights per unit volume.

One undesirable feature, but one not difficult to overcome, is the high initial pH produced by the buffer. This pH varies between levels of 9.2 and 9.8, depending upon the amount used. It is necessary to back titrate solutions to desired pH levels prior to use. We have employed concentrated hydrochloric acid for this purpose since chloride ion is already present in sea water in high concentration. Other acids, such as sulfuric or citric could be used. A portable pH meter can be used to measure final pH in the field. However, by adding known amounts of acid and buffer, the desired pH can be closely approximated without a pH measuring device. Both liquid and dry acids were tested (HCl and citric). This method of pH adjustment has proven reliable; the error of back titration amounting to a maximum of 0.05 pH units in our tests. Buffer and dry acid could be premixed for field use and packaged in known amounts (Table 1). It is

[3] Obtainable from the Sigma Chemical Co., 3500 DeKalb St., St. Louis 18, Mo.

TABLE 1

Cubic Centimeters of Concentrated HCl and Grams of Dry Citric Acid Necessary to Back-titrate Various Concentrations of Tris-buffer in Sea Water to a pH Between 8.0 and 8.5. The Recommended Concentrations Are Boldface.

| pH | Concentration of Tris-buffer in grams/gal. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2 | | 5 | | 10 | | 15 | | 20 | |
| | cc. HCl* | grams citric acid | cc. HCl | grams citric acid | cc. HCl | grams citric acid | cc. HCl | grams citric acid | cc. HCl | grams citric acid |
| 8.5 | 0.45 | 0.37 | 1.65 | 1.45 | 3.12 | 5.60 | 4.60 | 12.55 | 6.05 | 13.80 |
| 8.4 | 0.51 | 0.40 | 1.95 | 1.65 | 3.60 | 6.08 | 5.13 | 13.05 | 6.35 | 14.48 |
| **8.3** | **0.57** | **0.55** | **2.15** | **1.85** | **4.04** | **6.45** | **5.70** | **13.65** | **7.75** | **15.28** |
| **8.2** | **0.64** | **0.63** | **2.35** | **2.00** | **4.42** | **6.95** | **6.40** | **14.11** | **8.59** | **15.80** |
| 8.1 | 0.70 | 0.71 | 2.50 | 2.18 | 5.02 | 7.25 | 6.97 | 14.55 | 9.43 | 16.38 |
| 8.0 | 0.82 | 0.79 | 2.65 | 2.35 | 5.23 | 7.45 | 7.50 | 14.90 | 10.17 | 16.93 |

* pH should be measured if possible.

recommended that an end point between pH of 8.20 and 8.30 be sought for normal salt water transport. This will allow enough latitude to absorb small measuring errors without danger to the fish, while maintaining high buffering capacity. Table 1 indicates the amounts of HCl (concentrated) or citric acid (dry) required to reduce pH to various levels in one gallon of sea water after addition of a known amount of tris-buffer. By multiplying this figure by the number of gallons in a transport tank and adding the resulting amount of acid and buffer the desired pH can be approximated. Experience has shown that the allowable pH range during salt water transport lies between 7.5 and 8.5. The pH range for fresh water is much wider than this, as normal fluctuations are greater and fresh water fish are generally hardier to lower pH.

The buffer should be added and back titrated before fish are introduced to insure final adjustment of pH. If the compound is to be used in tanks with a recirculation system, the buffer and acid should be added and the water recycled to produce a stabilized pH before the fish are introduced.

When fish are to be shipped by air in plastic bags, concentrations of five grams per gallon will generally produce sufficient buffering. The actual amount of buffer which will be consumed during any transport operation is dependent upon the weight of fish per unit volume of water, the total metabolic activity, the initial pH, the natural buffering of the transport water, the temperature, and the time during transport. It is felt that the major factor contributing to pH decline resulting from this complex is carbon dioxide. Tris-buffer readily "absorbs" carbon dioxide and therefore should prove efficient in stabilizing pH in any system which tends to accumulate this gas. Because of the complex nature of pH change it is best to rely upon periodic measurement during transport.

The data indicate that tris-buffer is nontoxic to 29 species of fishes (Table 3). Other forms should be tested prior to transport.

## THEORETICAL AND EXPERIMENTAL BACKGROUND

### Causes of Mortality During Transport

Understanding the factors which cause death or distress to fishes during transport is basic to improving present transport methods. Several variables can become lethal agents during transportation. These can act individually, or more likely, in combination, and thus with increased possibilities of causing mortality. The most obvious factors which may become lethal are: temperature, oxygen tension, waste product levels, carbon dioxide tension, and pH.

#### *Temperature*

In general, increasing temperatures produce difficulties during transport primarily because rates of metabolism are increased and other parameters thus tend to become critical.

#### *Oxygen*

Most species of fish do well when the tensions are above 2.0 cc $O_2$/liter. Under extremely crowded conditions however, Haskell (1940)

has shown that tensions above 8-10 cc $O_2$/liter are required by trout. Such high requirements are possibly related to the existing level of $CO_2$, in addition to possible effects of increased activity.

### *Waste Products*

The accumulation of wastes from the metabolism of fishes can become a serious problem. Brockway (1950) points out that most wastes excreted through the gills consist of $NH_3$, urea, and amino oxides, while creatine and uric acid are excreted by the kidneys. In addition to these materials the production of mucus and its liberation into the water and regurgitated food material can contribute to pollution. Ellis, Westfall, and Ellis (1946) have shown the extreme toxicity of ammonia to certain fish species even at levels as low as 1.0 part per million (p.p.m.). In his experiments upon survival of *Tilapia mossambica* in sealed jerry cans, Vaas (1952) found that the bacterial counts of the medium tended to increase exponentially with time. This increase in bacteria was supposedly caused by an increase in the organic content of the water from metabolic wastes. Phillips and Brockway (1954) found that the ammonia content rose to 25 p.p.m. during 12 hours in aquaria containing brook trout at high density. Placing the fish in cooler water, prolonged starving and treating starved fish with sodium amytal, all caused significant declines in the accumulation of ammonia during 12 hours. The results from these experiments are inconclusive, but indicate that accumulation of waste products may reach lethal levels in transport under extreme conditions. As yet no completely adequate agent has been developed to control ammonia, but Saha, Sen, and Mazumdar (1956) report that several resins have the ability of absorbing it. A product called Amberlite proved to be the most useful. Nemoto (1957) has also tested resins. His results show Amberlite-IR-120 to be useful in the absorption of nonprotein nitrogen ($NH_3$).

### *Carbon Dioxide and pH*

The anaesthetic effects of $CO_2$ have long been known. Its accumulation during transport has been controlled by providing adequate gas exchange, through aeration. Fish and Hanavan (1948) indicate that during transport of salmon around Grand Coulee Dam, the pH fell from 7.8 to 6.4, while the $CO_2$ rose from three to four p.p.m. to a high of 17-18 p.p.m. during the four hours of hauling. These figures are important in that the transport water was continually aerated and the $O_2$ concentration, after an initial decline, returned to a normal level of seven p.p.m. We have noticed similar changes during transport of marine fishes over distances as great as 1,000 miles. During one such trip (Guaymas, Mexico, to Los Angeles, California) the pH declined during the first hour from 8.20 to 7.40. The addition of a second circulation pump was only sufficient to maintain this lower pH. Oxygen was always at normal levels. It is evident that even with aerating systems capable of maintaining ample quantities of dissolved $O_2$, $CO_2$ may accumulate to high levels and result in a marked decline in pH.

In addition to the narcotic action of $CO_2$, a fall in pH can produce other serious effects. Townsend and Cheyne (1944) demonstrated that increasing acidity (from several acids) can cause mortality. Further changes in both $CO_2$ and pH have serious effects on fish haemoglobins.

## Mortality in Closed-system Transport

In recent years pure $O_2$ has been employed in the importation of both fresh water and marine fishes. It is usually applied by placing the fish in a polyethylene plastic bag and displacing the air over the water with $O_2$. The bag is sealed, cartoned, and shipped to its destination by air.

The use of oxygen in fish transport was apparently first employed in Europe in the last part of the 19th century (Shebley, 1927). Osburn (1910) reports the use of $O_2$ in fish transport and since that time several papers have appeared regarding its use (Wiebe and McGavock, 1932; Haskell, 1940; Mitra, 1943; Khan, 1946; Sundara and Cornelius, 1949; Basu, 1949, 1952; Vaas, 1952; and Saha, Sen, and Mazumdar, 1956). Of these papers only the works of Basu, Vaas, and Saha, et al. make any attempt to relate the use of $O_2$ to mortality and the weight of fish per unit volume which can be transported.

At Marineland of the Pacific we have employed this method to import fishes from the Hawaiian Islands. Because of the air freight charges, resulting almost entirely from the poundage of shipping water which must be used, the method is expensive. Therefore it is necessary to keep the weight of fish per unit volume as high as possible. As a result mortality was often high on arrival and many bags contained fish in poor condition, exhibiting severe upsets in equilibrium. It was decided to determine, if possible, the causative agent of mortality and loss of equilibrium. Subsequent shipments were analyzed for dissolved $O_2$ and pH.

Oxygen analyses revealed that the oxygen tensions in shipping water upon arrival were more than ample to maintain metabolism under normal conditions. Oxygen tensions never fell below a value of 6.60 cc $O_2$/liter even though in a few instances mortality of all fish in the bag had occurred. Oxygen was determined by the sodium azide modification of the Winkler method.

Seventy-one bags were analyzed for pH upon arrival. Initial pH values in Hawaii were grouped around a value of 8.20. A mortality of 31.9 percent of all fish occurred. In contrasting the pH values for bags containing live fishes against those in which mortality had occurred (mortality considered as 25 percent or more dead animals in a bag), it was found that a significant difference in pH existed (Table 2). Measurements of pH were made with a Beckman pH meter (Model N).

## Use of Buffers to Control pH

The results suggest that increasing acidity and $CO_2$ could have been the major factors involved in mortality. If these factors were not the actual causative agents, they are correlated with mortality. The increases in acidity are attributable to the accumulation of $CO_2$ and waste products. Since pH in most instances fell drastically, whether death occurred or not, it was felt that the addition of a buffer to the

TABLE 2
Analysis of pH of Plastic Bag Transport Water Containing Fishes Shipped From Hawaii

| Condition of fish | Number of bags | Mean pH upon arrival | pH range |
|---|---|---|---|
| Alive | 48 | 7.03 | 6.60–7.92 |
| Dead* | 23 | 6.78 | 6.42–7.15 |

(Difference between means: $t_{0.99} = 2.37$, $t = 58.1$, $P < 0.01$)
* Bags containing more than 25 percent dead specimens are considered in this group.

medium would be beneficial. Since $O_2$ never sank to normal lethal levels, control of pH was expected to result in an increase of time during which fish could survive in the bags.

### *Inorganic Buffers*

Several attempts have been made to stabilize pH during fish transport by use of inorganic buffering compounds. The results from the Hawaiian shipments agree with the findings of Vaas (1952) who showed that mortality of *Tilapia mossambica* in sealed jerry cans, was associated with increasing $CO_2$, and not with falling $O_2$ tensions. He found that the addition of sodium monophosphate ($Na_2HPO_4$) to the water at concentrations from 1.5-3.0 grams per liter increased the time to mortality by an average of about 40 percent. Srinivasan, Chacko, and Valsan (1955) report that the mortality of carp fry, after 48 hours in sealed cans was 2 to 4 percent in $Na_2HPO_4$ buffered water, while it was 10 percent in nonbuffered water. In open containers sodium monophosphate did not produce differences in mortality. Saha et al. (1956), report opposite findings using cyprinid spawn. They found a decrease in the time to mortality of 37 percent upon the addition of sodium monophosphate. They suggest that the addition of a single component cannot explain the increase in time to mortality reported by Vaas. They point out that in buffer theory the equilibrium relationship existing between a salt and a weak acid or base determines the buffering capacity. Although only one component was added by Vaas (op. cit.), other ions were present as indicated by the initial alkalinity figures, and these could give the necessary weak acid-salt ratio required by theory. Further, the negative results reported by Saha et al., might reflect a sensitivity of cyprinid spawn to the buffer. Nevertheless, the rate of pH decline in Vaas's buffered groups was lower than in controls. Further, the nonbuffered groups could not utilize the available oxygen by reducing its tension as low as the buffered groups. This suggests that the presence of sodium monophosphate considerably decreased the $CO_2$ concentrations. The relationship of $CO_2$ to mortality may be correlated with its effect on the $O_2$ loading tensions of the blood. The results of Vaas and Srinivasan et al. (op. cit.), are at variance with those of Saha et al. (op. cit.); therefore the value of using phosphate buffers in the transport of fresh water fishes remains in doubt. Nemoto (1957) reports doubtful utility of $Na_2HPO_4$ in transport of fishes.

Sea water, in comparison to most fresh waters, has a higher buffering capacity (Sverdrup, Johnson, and Fleming, 1942, pp. 195 and 202). This action is due to the presence of carbonate, phosphate, and borate salts. Sea water absorbs about seven times as much hydrogen ion as distilled water when the pH is back-titrated to 4.50. The buffering capacity of sea water seems insufficient to absorb the quantity of acidic substances given off by fishes during normal transport. Therefore, it was attempted to increase this capacity by utilizing the salts known to be present in sea water. Sodium carbonate and sodium bicarbonate were tried at various concentrations. Sodium carbonate, above a concentration of five mM (two grams per gallon), caused precipitation of calcium and magnesium and was therefore considered undesirable. This precipitate became quite heavy at concentrations above 10 mM (five grams per gallon). Sodium bicarbonate did not cause precipitation at concentrations up to 63 mM (20 grams per gallon). It showed marked buffering at concentrations from 15 mM to 63 mM. The amount of acid necessary to reduce the pH to a value of 4.50 was 7.4 and 30 times as great respectively, as the amount required in untreated sea water (Figure 1).

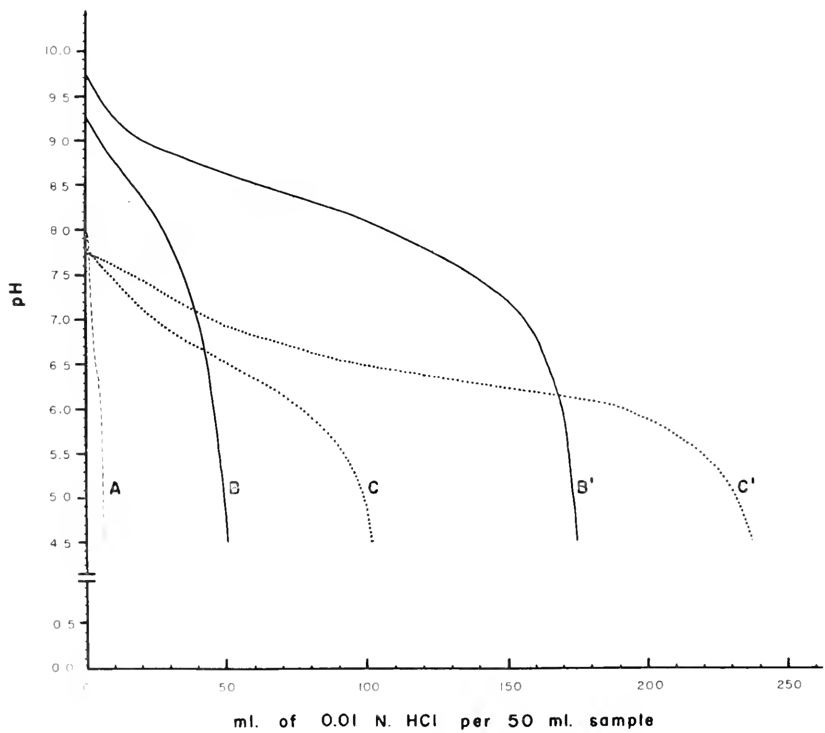



FIGURE 1. Titration curves for 50 ml. samples of sodium bicarbonate, tris-buffered, and untreated sea water. A, untreated sea water; B, tris-buffer, 5 gms. per gallon; B′, tris-buffer, 20 gms. per gallon; C, sodium bicarbonate, 5 gms. per gallon; C′, sodium bicarbonate, 20 gms. per gallon. The stippled area between pH of 7.5 and 8.5 represents a recommended range for transport of marine fishes.

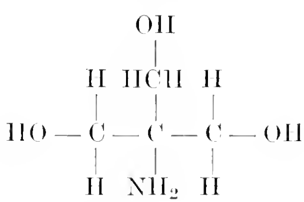
In Figure 1, curves C and C′ represent the amount of 0.01 N HCl required to reduce the pH to 4.50 in two 50 ml. samples, when the concentrations of sodium bicarbonate in sea water were increased by 15 and 63 mM respectively. Curve A shows the titration curve for untreated sea water. The curves show clearly that the buffering capacity is markedly increased, but the pH range at which the greatest hydrogen ion absorption occurs is low. This pH range, lying between 6.0 and 7.0, has already been associated with mortality of marine fishes in plastic bag transport.

The recommended pH range (8.5-7.5) is represented by the stippled area in Figure 1. The buffering capacity of sodium bicarbonate is very low in this area. For this reason we turned our attention to other buffers with high capacities, but in the desired range. Several concentrations of sodium monophosphate and sodium phosphate were tried. These, like sodium carbonate, caused heavy precipitation of calcium and magnesium and were eliminated.

### *Organic Buffers, Tris-buffer*

In discussing this problem with Dr. Thomas W. James of the Zoology Department of the University of California at Los Angeles, biochemical buffers, including tris-buffer were suggested. The latter compound is used to stabilize pH between 7.0 and 9.0 in tissue culture and enzymological studies. It has many properties applicable to the transportation of fishes. It does not cause any precipitation in sea water. Solutions are stable for periods up to three months at normal air temperatures while the dry salt is stable for long periods at temperatures up to 100 degrees C. The manufacturer reports that it is inert with respect to enzyme systems tested (Technical Bull. No. 106, Sigma Chemical Co. November, 1955).

Tris-buffer (2-amino-2-hydroxymethyl-1,3-propandiol) is a white crystalline solid with a molecular weight of 121.136. Its structural formula is:



It is a weak base with a dissociation constant of $1.202 \times 10^{-6}$ at 25 degrees C. Titration curves for this compound at concentrations of 10.95 mM (five grams/gallon) and 43.8 mM (20 grams/gallon) are shown in Figure 1 (curves B and B′). The buffering capacity is approximately the same as sodium bicarbonate at equivalent concentrations. However, the maximum absorption of hydrogen ion occurs between 7.50 and 8.50. At a buffer concentration of 10.95 mM, buffering capacity is increased 15-fold, while at 44 mM it is increased 50-fold, compared to untreated sea water.

Oxygen analyses were performed on solutions containing various concentrations of tris-buffer in salt water to determine if its presence

had any effect upon the solubility of $O_2$. No significant differences in solubility levels were obtained. Tris-buffer therefore appeared to offer a possible solution to the difficulties encountered in the use of inorganic buffers.

*Effects of Tris-buffer.* Twenty-nine species of fishes were exposed to tris-buffer to determine if harmful properties existed. These species represent 16 different families. All but four species were marine forms. These data are summarized in Table 3. In several cases fish exposed to the buffer for two days were placed in untreated water and observed for 30 days. Increases in weight occurred in all fishes which were examined. Fresh water species were left in buffered solutions for nine days. The pH decline during this period was 0.02 pH units. One specimen of a cichlid (*Aequidens portalegrensis*) died during the third day of the experiment. This death was probably due to starvation since none of the fishes were fed until the fourth day. No other mortality occurred during the course of the experiment.

Although the number of species we have exposed to tris-buffer is small the compound appears to be nontoxic and does not cause serious stresses. However, caution should be used in its application to new forms.

TABLE 3
Effects of Tris-buffer on Various Species of Fish

| Species | Number of specimens | Buffer (gms/gal) | Initial pH | Exposure (hours) | Condition after exposure |
|---|---|---|---|---|---|
| Marine Species | | | | | |
| *Heterodontus francisci* | 1 | 20 | 8.29 | 48 | Normal at 30 days |
| *Holocentris microstomus* | 2 | 2 | 8.25 | 12.5 | Normal on arrival |
| *Myripristis murdjan* | 1 | 2 | 8.25 | 12.5 | Normal on arrival |
| *Pseudupeneus bifasciatus* | 1 | 2 | 8.25 | 12.5 | Normal on arrival |
| *Amphiprion percula* | 2 | 20 | 8.39 | 48 | Normal at 30 days[2] |
| *Lepidaplois bilunulatus* | 1 | 2 | 8.25 | 12.5 | Normal on arrival |
| *Iniistius pavo* | 1 | 2 | 8.25 | 12.5 | Normal on arrival |
| *Girella nigricans* | 9 | 20[1] | 8.41 | 48 | Normal at 30 days[3] |
| *Kuhlia marginatus* | 4 | 2 | 8.25 | 12.5 | Normal on arrival |
| *Hippocampus punctulatus* | 1 | 20 | 8.39 | 48 | Normal at 30 days[4] |
| *Hepatus olivaceus* | 2 | 2 | 8.25 | 12.5 | Normal on arrival |
| *Hepatus barieue* | 1 | 2 | 8.25 | 12.5 | Normal on arrival |
| *Microcanthus strigatus* | 1 | 2 | 8.25 | 12.5 | Normal on arrival |
| *Zebrasoma flavescens* | 2 | 2 | 8.25 | 12.5 | Normal on arrival |
| *Zanclus canescens* | 1 | 2 | 8.25 | 12.5 | Normal on arrival |
| *Heterostichus rostratus* | 1 | 20 | 8.29 | 48 | Normal at 30 days |
| *Leptocottus armatus* | 2 | 20 | 8.29 | 48 | Normal on arrival |
| *Clinocottus analis* | 1 | 20 | 8.29 | 48 | Normal on arrival |
| *Balistes vidua* | 1 | 2 | 8.25 | 12.5 | Normal on arrival |
| Fresh Water Species | | | | | |
| *Lebistes reticulatus* | 16 | 10 | 8.40 | 9 days | Normal |
| *Mollienesia* sp. | 2 | 10 | 8.40 | 9 days | Normal |
| *Aequidens portalegrensis* | 5 | 10 | 8.40 | 9 days | Normal (1 dead) |
| *Cichlasoma nigrofasciatus* | 3 | 10 | 8.40 | 9 days | Normal |

[1] Interrupted opercular movements.
[2] Mean weight gain, 27.5 percent.
[3] Mean weight gain, 9.5 percent.
[4] Mean weight gain, 11.0 percent.

*Respiratory Effects.* Specimens of the opaleye (*Girella nigricans*) exhibited a marked interruption of opercular movements after several hours in tris-buffer. Normal opercular rates in small specimens (40-80 mm.) vary from 120 to 200 beats per minute at 17-18 degrees C. Irregularities appear after five hours exposure to tris-buffer at 5 to 20 grams per gallon. These irregular movements are characterized by two or three large opercular beats, followed by a pause lasting 5 to 10 seconds. Total beats varied from 8 to 20 per minute. This upset in opercular rhythm does not seem to have an adverse effect upon the fish. The nine fish listed in Table 3 all showed this pattern. They seemed normal in all other respects. The cause of the respiratory interruption is undetermined. It is possible that $CO_2$ or pH is effective in the control of the respiratory rate in opaleye. The presence of the buffer might change values of these factors, resulting in disturbance of the respiratory center. Such a mechanism exists in other vertebrates (Prosser et al., 1950, p. 254; Babák and Dedek, 1907; Hyde, 1904; Leiner, 1938; and Willmer, 1934).

## Use of Tris-buffer in Open System Transport

Tris-buffer is applicable to aerated transport systems. A pH decline in an open system has already been shown for fishes carried from Guaymas, Sonora, Mexico, to Los Angeles, California. The results reported by Fish and Hanavan (1948) on salmon transport have been previously discussed. It is probable that $CO_2$ accumulation is a critical factor in almost all present day transport operations and should serve as a guide to the design of efficient aerating devices. Most fish transportation systems and techniques have been developed empirically and probably succeed by staying somewhat above lethal $CO_2$ levels.

The effects of tris-buffer were tested in an aerated fish transport tank during normal operation. This test was performed through the courtesy of Steinhart Aquarium, San Francisco, California. A shipment of tropical marine fish arrived in Los Angeles and were transported to San Francisco by truck. Four small tanks were used. One tank was treated with tris-buffer at a concentration of two grams per gallon and the pH back-titrated to 8.25. The results are indicated in Table 4. The pH declined and the $CO_2$ increased in all tanks except the buffered tank (No. 4). The loads transported were small and no mortality occurred. The results suggest that tris-buffer may be extremely valuable in transportation of fishes where heavy loads or long distances are involved.

TABLE 4
The Use of Tris-buffer in Stabilizing pH and $CO_2$ in Open System Transport Tanks

| Tank No. /(gals.) | Tris-buffer in gms. /gal. | Total lapsed time in hours | Initial pH | Final pH | Difference in pH | Initial $CO_2$ in ppm | Final $CO_2$ in ppm | Difference in $CO_2$ in ppm |
|---|---|---|---|---|---|---|---|---|
| 1/(40) | -- | 12.5 | 7.95 | 7.60 | —.35 | $1.5\pm.2$ | $3.52\pm.2$ | 2.02 |
| 2/(90) | -- | 12.5 | 7.95 | 7.40 | —.55 | $1.5\pm.2$ | $2.62\pm.2$ | 1.12 |
| 3/(90) | -- | 12.5 | 7.89 | 7.40 | —.49 | $2.0\pm.2$ | $4.40\pm.2$ | 2.20 |
| 4/(40) | 2.0 | 12.5 | 8.25 | 8.20 | —.05 | None | None | 0.00 |

## Tests Performed With Tris-buffer in the Laboratory

Several pilot experiments were performed to determine the suitability of tris-buffer for plastic bag transport. In these experiments fishes were sealed in bags with pure $O_2$ over a buffered solution. The results were encouraging but technical difficulties did not allow rigid control of the experiments. We were not able to draw periodic water samples or to regulate the volume of the gas phase.

### *Method*

To overcome these difficulties we built a simple apparatus which simulated a plastic bag. It consisted of a series of five-gallon carboys fitted with rubber stoppers, into which a siphon, a manometer, and a stopcock were inserted. A standard medical $O_2$ cylinder was connected to the stopcock. By use of the manometer it was possible to maintain a virtually constant $O_2$ pressure inside the carboys. The siphon allowed withdrawals of water samples. Temperature was controlled by placing the carboys in a 40-gallon tank through which water of a constant temperature was circulated.

Three gallons of water were introduced into each carboy and the air initially displaced with pure $O_2$. Fish were placed in each vessel and the stoppers inserted. A layer of mineral oil was poured into a well created by pushing the stoppers down into the neck of the carboy, to assure a seal. *Fundulus parvipinnis* was chosen as the experimental animal. It is extremely hardy and was expected to survive conditions which would readily kill a more sensitive fish. Weights of all samples of fish were measured by water displacement prior to placement in the vessels. They were weighed individually at the end of the experiment.

During the course of the experiment periodic determinations of pH, $O_2$, alkalinity, and turbidity were made. The total water withdrawal from each vessel amounted to 10.9, 17.8, 19.5, 23.6 and 12.9 percent of the total volume in vessels one through five respectively. The percentage decrease between consecutive readings was small (less than 1 percent of total volume), although the total volume reduction was considerable. Since the increment of change was slight it should have had little effect upon the trends shown by the data. However, end points may have been somewhat displaced. At the beginning and end, inorganic and organic $N_2$ concentrations were determined by the Kjeldahl method. Carbon dioxide was indirectly determined by use of the Tillman formula, which expresses the relationship existing between pH, alkalinity and salinity (Quinn and Jones, 1936). This equation is:

$$pH = \log \frac{(\text{alkalinity} \times .203 \times 10^7)}{(CO_2)}$$

The alkalinity is expressed in p.p.m. as $CaCO_3$. Carbon dioxide is expressed in p.p.m. This relationship is assumed to hold in the presence of the high alkalinity titres produced by the presence of tris-buffer. No attempt (e.g. gasometric methods) was made to check this assumption. Carbon dioxide values obtained in tris-buffer solutions might therefore be subject to considerable error.

The decline in pH and time to 50 percent mortality are graphically presented in Figure 2, while the accumulation of $CO_2$ and time to 50 percent mortality are indicated in Figure 3. Because the sea water supply had a pH of 7.80 the buffered solutions were back-titrated to approximately this level, except in group five in which the pH was adjusted to 8.25.

## *Results*

Oxygen analysis showed that in all except two instances the tensions in the experimental jars were above 1.0 cc $O_2$/ liter. The jars lower than this were group one at 23 hours (0.82 cc $O_2$/ liter) and group four at 23 hours (0.62 cc $O_2$/ liter). Groups 1 through 5 are represented in the legend for Figure 2 and read from top to bottom respectively. These values are two to three times above the lower lethal limits for *F. parvipinnis* at pH levels of 7.0. Both groups were in good condition at 23 hours. The $O_2$ values at 50 percent mortality in groups one through five were 1.90, 3.0, 2.80, 1.70, and 2.80 cc $O_2$/ liter.

Turbidity was measured by nephelometry and results recorded in p.p.m. of $SiO_2$. In all cases the turbidity increased steadily with time. It tended initially to be linear in all groups and then increased rapidly before 50 percent mortality. The increases in turbidity agree with the results of Vaas (1952). Final values were 5.9, 28.8, 15.6, 14.5 and 20.2 p.p.m. $SiO_2$, for groups one through five respectively.

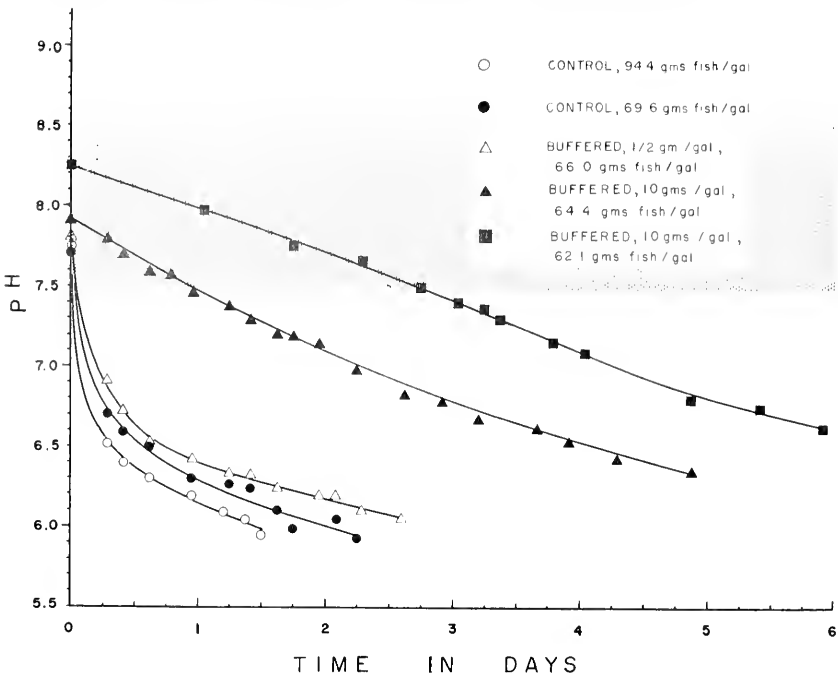



FIGURE 2. The effects of tris-buffer upon control of pH decline and time to 50 percent mortality in sealed containers using *Fundulus parvipinnis*. End points of each curve represent 50 percent mortality. The stippled area between pH of 7.5 and 8.5 represents a recommended range for transport of marine fishes.

Nitrogen analyses revealed considerable increases in inorganic and organic $NH_3$. The sea water source contained traces of free $NH_3$, while the organic fraction varied from a trace to about 0.01 p.p.m. The end points for inorganic $N_2$ in groups one through five were 1.5, 1.7, , 4.5 and 4.5 p.p.m. $NH_3$ respectively. The corresponding organic $N_2$ levels were 6.6, 14.0, 12.5, 63 and 103 p.p.m. $NH_3$ respectively. The Kjeldahl method is subject to error in sea water (probably at least 10 percent) so that the absolute values have little meaning. However, relative changes are evident and organic $N_2$ increases with time. The inorganic values reported in groups four and five possibly increased during distillation by some dissociation of the $—NH_2$ group present in the buffer molecule. The organic values have been corrected for the amounts of tris-buffer present and therefore reflect increases in organic wastes.

Figure 2 shows that tris-buffer decreased the rate of pH decline and at the same time markedly increased the time to 50 percent mortality. The group of fishes held at an initial pH of 8.25 (group five, black squares, Figures 2 and 3) showed a 2.84 fold increase in the time to 50 percent mortality compared to the control group (group two, black circles, Figures 2 and 3).

The groups which lived the longest died at the highest pH. This seems to be a result of prolonged exposure to low pH or high $CO_2$ concentrations. All death pH values are quite low. It is very probable that the majority of marine species exposed to similar conditions would have died at higher pH levels. *F. parvipinnis* is a euryhaline form frequenting habitats in which pH and $CO_2$ must vary widely.

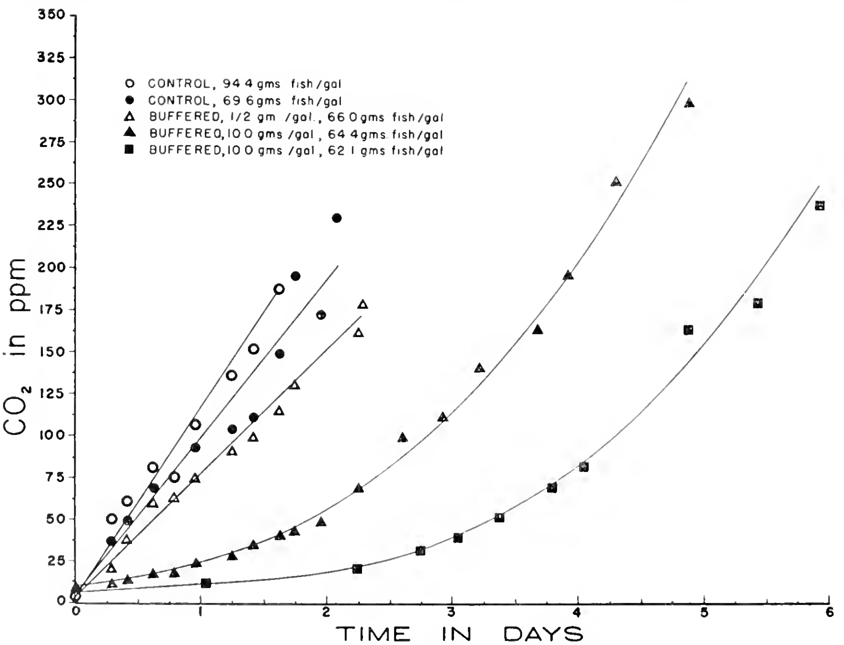



FIGURE 3. The effects of tris-buffer upon the control of $CO_2$ accumulation and time to 50 percent mortality in sealed containers using *Fundulus parvipinnis*. End points of each curve represent 50 percent mortality.

## DISCUSSION

The effects of metabolism upon the rate of pH decline in a closed system can be approximated theoretically by use of the Tillman Formula. To accomplish this a constant rate of $O_2$ uptake, a constant R. Q. (respiratory quotient), a constant production of $CO_2$, and a constant alkalinity in the medium must be assumed. Such assumptions are approximations since the rates involved can be affected by many variables. Discussions of the effects of numbers of fishes are found in Shlaifer (1938, 1939); of the effects of $O_2$ concentration, pH and $CO_2$ in Prosser, et al, (1950) and Fry (1957); of the effects of rhythms in Wells (1935) and Clausen (1936). However, assumptions of constant rates are probably not in serious error since our experimental results suggest that alkalinity and the rate of $CO_2$ production tended to be constant. Therefore, by choosing arbitrary weights of fish per unit volume, metabolic rates and R. Q.'s, the $CO_2$ production can be determined. By substituting this value in Tillman's Formula the pH at any time can be obtained. Figure 4 represents the pH decline for six groups of fishes which were calculated in this manner. Three weight groups (25, 50, and 225 grams of fish per gallon) and two metabolic rates for each weight (0.10 and 0.50 cc $O_2$/ gram body weight/hour) were chosen. The metabolic rates are based upon figures from Prosser, et al (1950) and represent values for sluggish and active fishes.

Several features important to closed system transport can be ascertained from a comparison of the properties of such theoretically derived curves, and the experimental curves shown in Figures 2 and 3.

First, the theoretical curves of Figure 4 demonstrate that even with low weights of fish per gallon, pH drops rapidly. It can be seen that the curve for the most sluggish form at the least weight per unit volume (curve six) drops to a pH of 7.5 in eight hours. Higher weights of fish per unit volume cause even faster declines in pH. This can be seen in the experimental control groups shown in Figure 2. Therefore, if considerable weights of fish are to be transported, the utility of buffering is apparent.

Second, pH does not decline as a linear function of time but tends to level out below a pH of 7.0. This trend is evident in both the experimental and theoretical curves. The nonlinearity is a direct function of the relationship of pH to $CO_2$ concentration, and is quantitatively expressed by the Tillman Formula. Therefore in closed system transport the pH decline is primarily a result of an interaction between the weight per unit of volume of fish and their metabolic rates. For instance, in Figure 4, curves two and five represent the same weight per unit volume (50 grams/gallon). The respiration rate for curve two is five times that for curve five. The time required to reach any pH level is five times as great in curve five as it is in curve two.

The control group in Figure 2 (black circles) had a mean $CO_2$ concentration which gives a calculated pH of 5.98 at 50 hours. Let us assume that an R. Q. of 0.8 and an $O_2$ metabolism of 0.20 cc $O_2$/gram/hour existed in this control group (these values are estimates calculated from Wells, 1935, p. 210, and Odum, 1956, p. 108). Then, substituting the total $CO_2$ concentration for 50 hours into the Tillman Formula, a

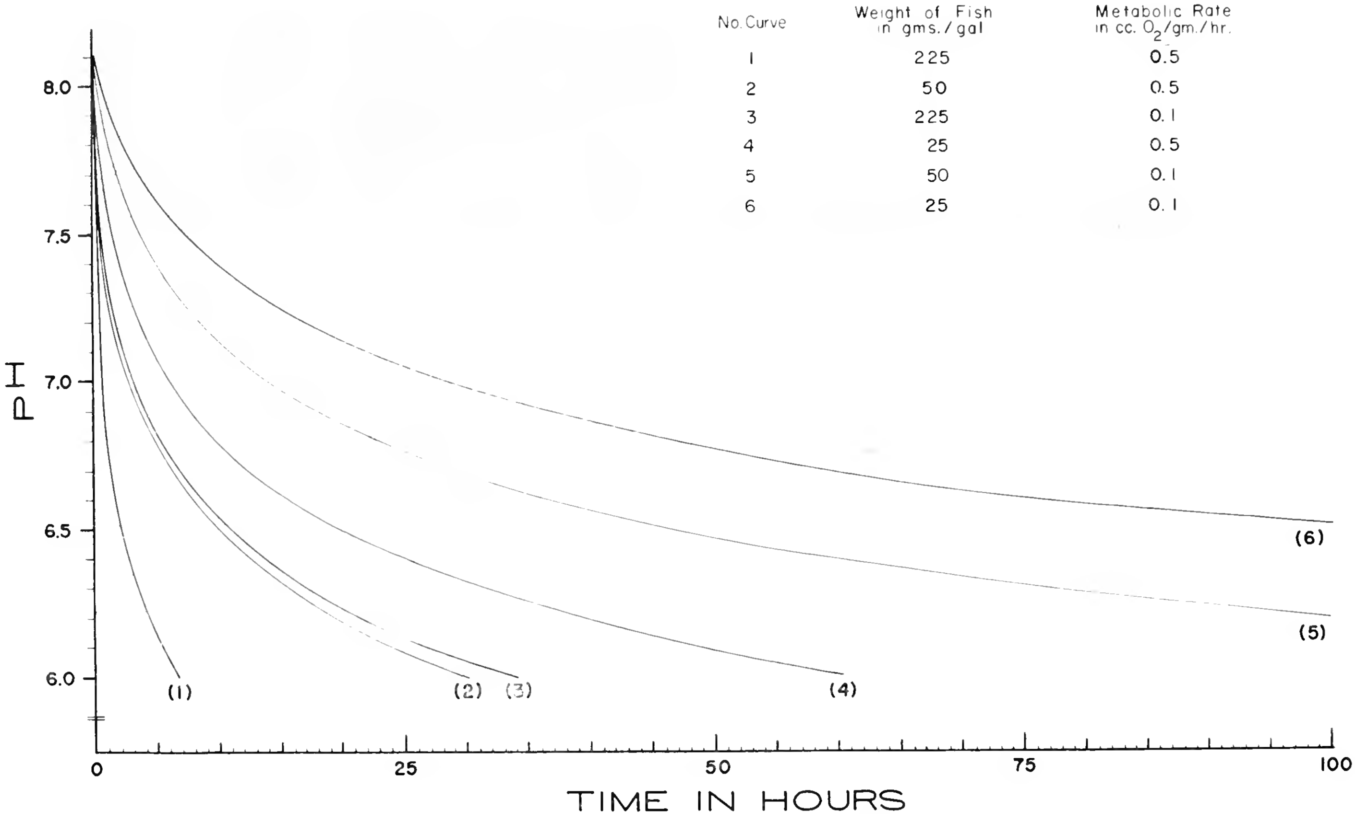



FIGURE 4. Hypothetical curves relating decline in pH to the weight and metabolic rate of fish due to accumulation of $CO_2$ in a closed system. The weights of fish are arbitrary. Metabolic rates represent sluggish and active type fishes. Carbon dioxide production was calculated for a respiratory quotient of 0.80. The stippled area between pH of 7.5 and 8.5 represents a recommended range for transport of marine fishes.

theoretical pH of 6.12 is obtained. The agreement between this value and the mean value of 5.98 is so close as to lend credence to the concept that the decline in pH in closed system transport is primarily due to the effects of $CO_2$ accumulation. The highly buffered groups (black triangles and black squares) do not exhibit this logarithmic decline in pH, but show a nearly linear decline.

Third, examination of the control groups and the lightly buffered group in Figure 3 show $CO_2$ accumulation at a relatively constant rate. This is in excellent agreement with the previous theoretical assumptions and supports the concept that $CO_2$ is the major factor causing increasing acidity. Although the $CO_2$ levels reported for the highly buffered groups are possibly in error, the accumulation follows trends which would be expected in the presence of a substance capable of buffering $CO_2$. The high $CO_2$ levels at 50 percent mortality (178-296 p.p.m.) are in agreement with the findings of Vaas (1952). He reported that mortality in *Tilapia mossambica* was the result of high $CO_2$ rather than $O_2$ depletion.

It was not possible to determine the actual cause of mortality from our data. Nevertheless, both in simulated and actual plastic bag transport, declines in pH and increases in $CO_2$ are correlated with mortality. High $CO_2$ levels and low pH's both cause a marked Bohr Effect in many fish bloods and in this manner seriously reduce the affinity of haemoglobin for oxygen (Krogh and Leitch, 1919; Root, 1931; Willmer, 1934; Prosser, et al, 1950, and Fry, 1957). Therefore, the $O_2$ tensions we have reported might well have been limiting due to the increased $CO_2$ concentrations present, even though they would have supported metabolism under normal circumstances. Further, several authors have shown that the ability of fishes to tolerate low $O_2$ tensions is dependent upon the $CO_2$ tension (Powers, 1922; Hall, 1931; Wiebe, et al., 1934; Meyer, 1935; van Dam, 1938; Safford, 1940; Hart, 1945; and Black et al., 1954). The effect of $CO_2$ tension on metabolic rate is incompletely known but there appears to be wide variation. Some fishes show marked changes upon slight increases in $CO_2$, while other species are highly tolerant to high $CO_2$ levels (Fry, 1957).

Whether $CO_2$ tension, pH or $O_2$ tension was actually responsible for mortality in our experiments is unimportant for control as long as the effects of their interactions are appreciated. The value of using tris-buffer to control this interacting system is evident.

In addition to its demonstrated buffering capacity, tris-buffer seems to retard bacterial decomposition of dead fish. In plastic bag shipments, buffered water was uniformly less turbid and foul smelling than untreated water, whether dead fish were present or not. The whole question of the effects of dead fishes upon transport water quality needs additional study.

The use of buffers affords an opportunity to increase the efficiency of fish transport. More data regarding their use in actual practice are needed. In addition, further improvements in transportation techniques can certainly be achieved by stabilization of other water quality parameters, such as waste product levels and ammonia.

## SUMMARY


(1) Chemical buffers are of importance in the control of acidity changes (expressed as pH) during fish transport, resulting from respiration and accumulation of waste products.

(2) A biochemical buffer, tris-hydroxymethyl-aminomethane (tris-buffer), has been found valuable for pH control, both in closed and open system fish transport.

(3) Inorganic buffers either caused heavy precipitation of salts in sea water or buffered outside the optimum pH range for marine fishes.

(4) Methods of application of tris-buffer in transport are discussed.

(5) A closed system experiment was carried out in which a constant pressure of $O_2$ was maintained over a volume of water containing a known weight of experimental fish (*Fundulus parvipinnis*). The lengths of time to 50 percent mortality for buffered and non-buffered groups were determined. Periodic water samples were tested for pH, $O_2$ concentration, $CO_2$ concentration, turbidity, inorganic and organic nitrogen. The most highly buffered group lived 2.84 times as long as the longest-lived control group.

(6) The interactions of $CO_2$, pH and alkalinity are discussed. A theoretical model relating the effects of metabolism upon pH, alkalinity and free $CO_2$ is developed. The model suggests that lethal limits will be rapidly reached if the weight of fish transported exceeds 25-50 grams per gallon in a closed system.

(7) The interactions of $CO_2$, pH and $O_2$ in relation to respiration of fishes are discussed. The correlation of high $CO_2$ concentrations and low pH levels with mortality is possibly due to the effects of these variables upon the ability of fishes to utilize oxygen.

(8) In open transport systems pH is shown to decline, while $CO_2$ increases, even during the use of aerating devices. Tris-buffer stabilized these factors in actual transport.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# AN ALL-PLASTIC DART-TYPE FISH TAG[1]

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## INTRODUCTION

The Pacific Oceanic Fishery Investigations (POFI), of the U. S. Fish and Wildlife Service, has been studying the skipjack (*Katsuwonus pelamis*) in Hawaiian waters as part of its program to assist the fisheries of the Pacific area. Two aspects of the biology of the skipjack on which there is presently little information are its migrations and growth. Tagging offers a direct means of obtaining information on these problems.

Employing the principle of the harpoon and utilizing plastic materials, a new fish tag has been developed which appears quite promising. The present model, patterned to some extent after the Woods Hole dart tag (through personal correspondence), has been designated the POFI D-2 tag.

Recoveries have been markedly higher than those from tags used in earlier large scale tuna tagging programs. For instance Godsil (1938) tagged 4,000 yellowfin tuna (*Neothunnus macropterus*) and skipjack with opercular disk tags and obtained no recoveries. The first successful tuna tag was developed by the California Department of Fish and Game (Wilson, 1953). This tag, in the form of a loop of plastic tied through the flesh immediately posterior to the base of the second dorsal fin, gave worthwhile returns on yellowfin tuna and albacore, but returns were low on skipjack. Our own experience is illustrative. Between 1954 and 1956, 1,961 skipjack, tagged in Hawaiian waters with the California tag, yielded 12 returns, including three from the stomachs of predators. This rate of predation may be attributed to the length of time required to attach the type G tag. As a result of its struggles during this period the skipjack is in a weakened condition when returned to the water. In this weakened condition skipjack tagged with the type G tag are more susceptible to predation than are skipjack tagged by a more rapid method. In contrast, 747 of 8,161 skipjack, that were tagged with dart tags in 1957, have been recovered, none in the stomachs of predators. Recoveries are still being made, and to date, the longest time at liberty has been 260 days. Recoveries from individual groups of 3,224 and 2,291 skipjack, tagged and released in 1957, equal 11 and 13 percent respectively.

The probable reasons for the success of the new tag are: (1) its application involves minimum handling of the fish; (2) it remains firmly anchored in the fish; (3) it is small and offers little resistance to the

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flow of water; and (4) it does not interfere with the fish's normal activity. An additional advantage is that the speed of application permits operators to release a maximum number of tags during the course of tuna live-bait fishing.

With respect to other fishes, the Wisconsin Conservation Department (personal communication) has conducted pond tests on lake sturgeon (*Acipenser fulvescens*) and found the tag better than other types used. They also obtained preliminary results suggesting the tag could be used on walleye (*Stizostedion vitreum*) and lake trout (*Cristivomer namaycush*).

## CONSTRUCTION

### Tag

The POFI D-2 tag (Figure 1) has two parts, a head with a barb and a streamer. The head is a Lucite rod, thirteen-sixteenths of an inch in length, and the streamer is a plastic tube five inches in length. The

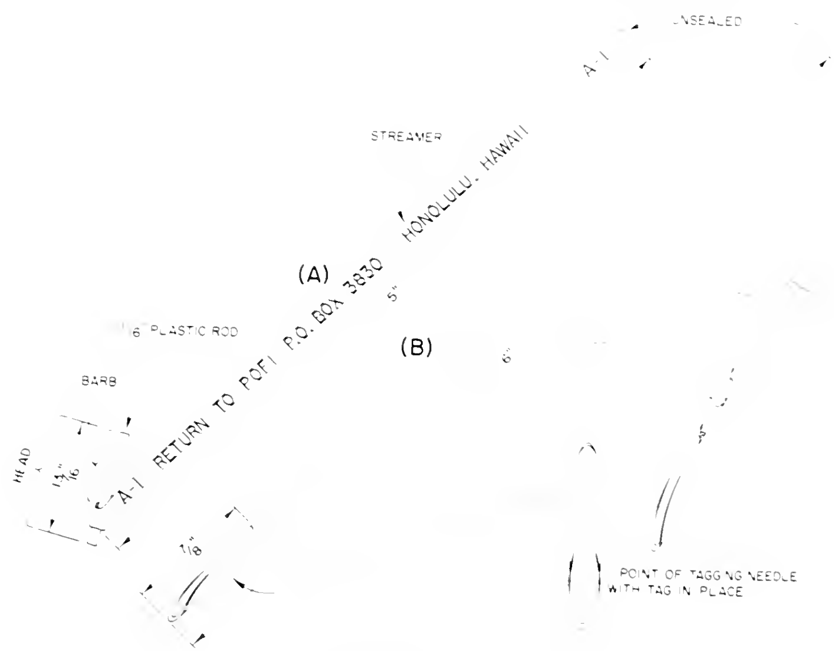


FIGURE 1. Diagrams of: (A) POFI D-2 tag and (B) tagging needle.

head is passed through a 45-degree cut made one-eighth inch from one end of the streamer, and these two parts are fastened together with a waterproof glue. A legend and serial number are printed on or contained within the streamer. The component parts and labor involved in fabrication of 1,000 tags is estimated at $95, while the cost of producing 100 needles approximates $57, but these latter are reusable and losses are minimal (Table 1).

TABLE 1
Components and Cost of POFI D-2 Tag and Needle

| Part | Description | Manufacturer or other source | Cost |
|---|---|---|---|
| Tag | | | |
| Head | Lucite rod ($\frac{1}{16}$ in. dia.) | Local plastics supplier | \$5.40, 1,000 tags |
| Streamer | Resinite EP-2 AWG size 13 | The Borden Company<br>Chemical Division<br>P.O. Box 1589<br>Santa Barbara, Cal. | \$5.85/1,000 tags |
| Waterproof glue | Seal-All | Allen Products Corp.<br>20450 Sherwood Ave.<br>Detroit 34, Mich.<br>(obtained from local hardware supply) | \$0.34, ¾-oz. tube |
| Legend | Machine printed, using vinyl plastic ink | Printing by:<br>Hugh C. McCoy<br>16400 Third Ave., SW.<br>Seattle, Wash. | \$25.00 +<br>\$0.225 (ink)/1,000 tags |
| Ink | Black vinyl plastic (No. 104N5A4) | California Ink Co.<br>546 Sansome St.<br>San Francisco, Cal. | \$1.35, 4-oz. bottle |
| Labor | Serial number and assembly | Mr. James Suyenaga<br>1172 Puu Poni St.<br>Pearl City, T. H. | \$55.00, 1,000 tags |
| Total cost/1,000 tags | | | \$95 |
| Needle | | | |
| Stock | No. 3 tempered stainless steel $\frac{5}{32}$ in. OD × .016 in. wall | Tubesales, Inc.<br>2211 Tubeway<br>Los Angeles 22, Cal. | \$54.00/100 ft.<br>(= 200 needles) |
| Labor | Cutting groove and sharpening point | Precision Industries<br>754 S. Queen St.<br>Honolulu, T. H. | \$30.00/100 needles |
| Total cost/100 needles | | | \$57 |

## Tag Modifications

The head on the present tag is blunt at both ends, whereas the earlier models had a sharpened barb to aid in firmly anchoring the tag. Sharpening the barb was found to be unnecessary.

The original streamer consisted of a clear plastic jacket, to which the head was attached, and a red or blue tubular plastic insert which bore the legend. A red, printed paper slip was later substituted for the plastic insert, and this gave a more readable legend. To protect the paper or plastic insert, the end of the outer jacket was plugged with a short piece of Lucite rod dipped in glue. The most recent modification of the streamer involves printing the legend and serial number, one at the base and another at the end of the streamer, directly on a red opaque tube, thus eliminating the insert and the plug. The red is preferred to the blue because the legend is more legible and the color is more obvious to the human eye.

Recovered tags have shown some fading of color and legend on the streamers and streamer inserts. This appears to have been caused by sunlight, because on many of the tags only the upper side of the protruding portion of the streamer is faded. The under side and the portion of the streamer inside the fish retained the color and legend. Efforts are being made to find an ink and a plastic that are less affected by sunlight.

### Tagging Needle

The tag is inserted with a stainless steel needle (Figure 1B) similar to that used for California type G tags. The point is cut as shown in the figure, so the tag head will be held securely. Without this feature the tip slips out of the groove, the head turns across the axis of the needle, and the streamer is cut off when the needle is inserted into the fish. The needle should be of sufficient length so that the end of the streamer does not protrude.

## TAGGING PROCEDURE

Before fishing begins, a number of needles are loaded with tags and bound in bundles of 10. On most POFI trips, 400-500 needles are so prepared. Each bundle is labeled with a slip of waterproof paper bearing the serial numbers of the tags. Since individual measurements of fish are not taken it is not necessary to identify individual tag numbers at the time of tagging. Instead the labels and the unused tags from a bundle of 10 are retained and checked after each fishing period or day to determine what numbers were released. A tagger can carry as many as 100 tags in the pockets of a canvas apron.

The D-2 tags have been employed principally in tagging skipjack caught by pole-and-line fishing. Tags of five-inch length have been used satisfactorily on fish ranging from 2 to 25 pounds. In the fishing operation, as commonly practiced by Hawaiian fishermen, skipjack are hooked, swung up, caught under the left arm, and unhooked. The tagger stands one to two feet behind the fishermen; ordinarily there is one tagger to two fishermen. After the skipjack is caught and while the hook is being removed, the tag-bearing needle is inserted usually from the right side, about one-half to one inch below the crest of the back and between the end of the second dorsal fin and the insertion of the first dorsal finlet. When the needle is removed, the barb holds the tag in place. The needle should form an angle of about 45 degrees with the body surface of the fish, and should be inserted with the barb next to the fish and deep enough so that the head of the tag crosses the median sagittal plane. The barb will then lodge against a neural spine and firmly anchor the tag in the fish. If the tag is inserted with the barb next to (Figure 2A), rather than away from (Figure 2B) the side of the fish, the tag will also be firmly anchored, even though the head does not cross the sagittal plane. The exact placement does not seem important as long as a major blood vessel is not injured. It is important that the end of the streamer does not reach the caudal fin, or the fin will be frayed by swimming movements of the fish. Small fish should be tagged anterior to the second dorsal fin in order to prevent this injury of the caudal fin.

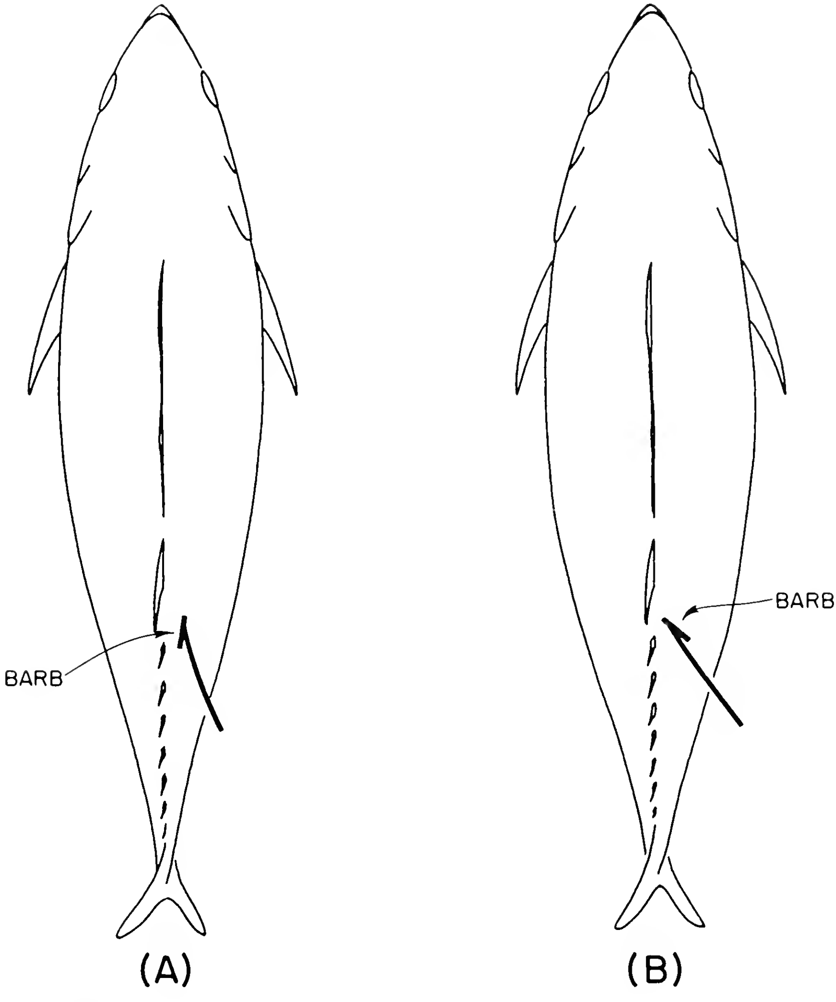


FIGURE 2. Diagrams showing dorsal view of tagged fish with barb inserted (A) next to and (B) away from side of fish.

Individual skipjack may be hooked, tagged, and returned to the water in as little as four seconds. Measurements of skipjack cannot be taken in this short time; instead, the size of tagged fish may be estimated from measurements of untagged fish caught from the same school. This estimate is considered to be reliable, because it has been shown that skipjack school by size (Brock, 1954).

## EFFECT OF TAGGING ON FISH

Underwater observations of tagged skipjack indicate that they are not greatly affected by the tagging procedure nor by the presence of the tag. Tagged fish were observed to sound, then return to the portion

of the school around the vessel. There have been seven instances in which tagged skipjack were recovered within minutes of being released. During the fall of 1957 one skipjack was tagged twice and released twice. The fish was initially tagged and released on November 20th. Three days later it was recovered by POFI fishermen and a second tag was inadvertently placed on it before it was again released. Fourteen days later this twice tagged skipjack was recovered by a commercial fisherman.

The tagging wound heals rapidly with no infection. Scar tissue is present within 10 days of tagging and well developed within a month. The scar tissue does not usually fuse to the tag but forms a tight, glove-like pocket around the head and streamer. The external wound is small, scarcely larger than the streamer (Figure 3).

## SUMMARY

An all-plastic, dart-type fish tag was used with considerable success on skipjack in Hawaiian waters during 1957. Recoveries from individual groups of 3,224 and 2,291 fish equalled 11 and 13 percent respectively. In addition to the high percentage of recovery the tag has

FIGURE 3. Skipjack with D-2 tag recovered 30 days after release. Tag pulled forward to show small wound and growth of new skin around opening. *Photograph by K. D. Waldron, June, 1957*

other desirable characteristics such as: (1) small size; (2) simplicity and rapidity of application; (3) low resistance to water flow; (4) secureness when attached properly; (5) negligible effect on the natural movements of the fish; and (6) one uniform size may be used on fish weighing between 2 and 25 pounds. Because of these advantages the dart tag should be suitable for use on a great variety of fishes.

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# CONDITIONS OF EXISTENCE, GROWTH, AND LONGEVITY OF BROOK TROUT IN A SMALL, HIGH-ALTITUDE LAKE OF THE EASTERN SIERRA NEVADA[1]

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## INTRODUCTION

Eastern brook trout (*Salvelinus fontinalis*) are introduced at fingerling size into many alpine lakes in California, on a population-sustaining rather than a put-and-take stocking basis. Although these trout are best appreciated by anglers for their readiness to take baits and lures, and for their quality in the frying pan, they are also favored by fishery managers for their ability to maintain themselves in such marginal situations as are frequently found in high-mountain lakes. Whereas other species generally require moving water and selected stream-bottom areas for spawning, brook trout are often able to reproduce by spawning on spring-fed areas of the lake bottom. This lake-spawning ability is an important factor in the maintenance of trout populations in drainages where some lakes, otherwise well qualified to support trout, have no interconnecting streams or have their tributaries channeled through broken rock in which there is little if any bottom suitable for spawning.

To learn more about the success and longevity of brook trout in a poor habitat at high altitude, the U. S. Fish and Wildlife Service in 1951 began a study of Bunny Lake, a snow-fed, 2½-acre cirque lake located at the upper limit of drainage (elevation 10,900 feet; U. S. Geological Survey, Mt. Morrison Quadrangle, 1953) in the northwest arm of Convict Creek Basin, Mono County, California. Being barren of fish before this study, Bunny Lake was not included in the earlier 1950-51 biological survey of 10 other lakes in the basin (Reimers, Maciolek, and Pister, 1955). A separate survey was therefore made in July, 1951, to include morphometry, summer temperature characteristics, chemical quality, and invertebrate fauna. In late August, 1951, approximately 1,800 brook trout fingerlings were packed in and planted in the lake. Observations and samples have been taken at intervals since that time. Because the lake is practically unknown, and is some distance from the trails that traverse this part of the drainage, it is considered unlikely that angling has disturbed this experimental population.

Reed S. Nielson, Chief of the California-Nevada Sport Fishery Investigations, Bureau of Sport Fisheries and Wildlife, directed the

[1] Submitted for publication February, 1958.

project. John A. Maciolek and Harry D. Kennedy, members of the staff, participated in the study. E. Philip Pister and Bobby D. Combs, former staff members now employed elsewhere, also assisted actively. Trout were provided by the California Department of Fish and Game.

## CHARACTERISTICS OF THE LAKE

### General Description

Bunny Lake is bounded on three sides by a high granite crest with associated talus, and on the fourth (outlet) side by the spillway of the small glacier that originally formed the cirque. Vegetation at the lake margin is limited to sparse grass and emergent dwarf rushes, and the immediate slopes support only a few shrubby lodgepole pines. The situation is shown in the photographs (Figures 1-3).

A snowfield, varying in size from year to year but always present to some extent all summer, spreads over a depressed part of the north-facing slope and impinges on the south shore. Melting snow and seepage, largely from the snowfield, maintain the lake at the level of its outlet. This permanence distinguishes Bunny Lake from numerous high-level snow pools of similar size and origin, particularly those on south-facing slopes or on cols, which become desiccated or greatly reduced in late summer as the snow disappears.

The foreshore consists of broken rock and bedrock interspersed with coarse sand, and provides many places of shelter for trout during the season of open water. Deeper bottom areas have the same rocky nature, but sand and some organic sediments cover most of the rock.

FIGURE 1. View of the rock basin (top center) which contains Bunny Lake. Water in foreground is Cloverleaf Lake. *Photograph by E. P. Pister, July, 1951.*

FIGURE 2. A closer view of Bunny Lake and the south slope of its basin.
*Photograph by E. P. Pister, July, 1951.*

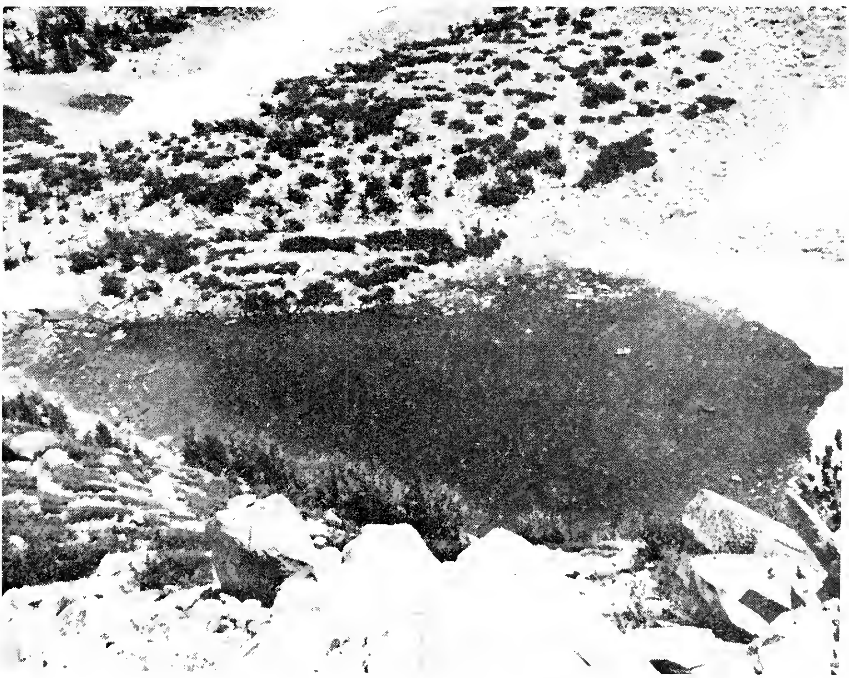

FIGURE 3. Plan view of Bunny Lake from a high point to the west.
*Photograph by E. P. Pister, July, 1951.*

## Physical Features

The lake is heart-shaped, as seen in Figure 3, with the attenuated end toward the outlet at the north. The map (Figure 4), which was prepared on a plane table by the stadia method to include 112 soundings, illustrates the division of shoal and deeper areas. The following morphometric information was derived from basic measurements, planimeter data, and standard computation.

| | | | |
|---|---|---|---|
| Surface area | 2.54 acres | Maximum length | 538 feet |
| Maximum depth | 24.5 feet | Maximum width | 362 feet |
| Mean depth | 6.8 feet | Length of shoreline | 1,256 feet |
| Volume | 17.34 acre-feet | Shore development | 1.07 |
| Mean slope | 13.8 percent | | |

Shore development is the ratio of the actual perimeter of the lake to the minimum (circular) perimeter of the same area. A value near unity, as above, indicates that the shoreline is almost completely free of embayments and irregularities.

As in other lakes of the area, the water of Bunny Lake has a high degree of transparency at most times, due to the scarcity of suspended matter. Exceptions may occur in such a small lake during periods of extreme wind agitation or at times of rapid drainage in early summer. In the initial survey, and in later tests, a 10-centimeter Secchi disk was easily visible through the greatest depth of water.

Water temperatures were recorded at each visit to the lake, but no attempt was made to establish a complete temperature cycle in any summer. Representative midday temperature structures for July and August appear in Table 1. Daily variations at midsummer, amounting to several degrees near the surface, are shown in Table 2. The important

TABLE 1
Some Summer Water Temperatures at Bunny Lake

| Depth in feet | Temperature in degrees F. at midday on | | | |
|---|---|---|---|---|
| | 7/16/51 | 7/19/51 | 8/1/51 | 8/29/57 |
| 0 | 54.6 | 51.0 | 55.0 | 44.6 |
| 2 | 52.3 | 51.6 | 54.0 | 44.6 |
| 6 | 49.8 | 49.3 | 53.4 | 44.6 |
| 10 | 49.3 | 49.1 | 53.1 | 44.4 |
| 14 | 49.1 | 48.9 | 52.9 | 44.2 |
| 18 | 48.9 | 48.7 | 52.8 | 44.2 |
| 22 | 48.9 | 48.6 | 52.7 | 44.2 |

TABLE 2
Daily Variations in Water Temperature at Bunny Lake

| Depth in feet | Temperature in degrees F. at | | | |
|---|---|---|---|---|
| | 7.30 p.m. 7/31/51 | 7.00 a.m. 8/1/51 | Noon 8/1/51 | 3.30 p.m. 8/1/51 |
| 0 | 58.3 | 52.7 | 55.0 | 57.9 |
| 6 | 53.6 | 52.7 | 53.4 | 53.8 |
| 14 | 53.2 | 52.7 | 52.9 | 53.2 |
| 22 | 52.7 | 52.7 | 52.7 | 53.2 |

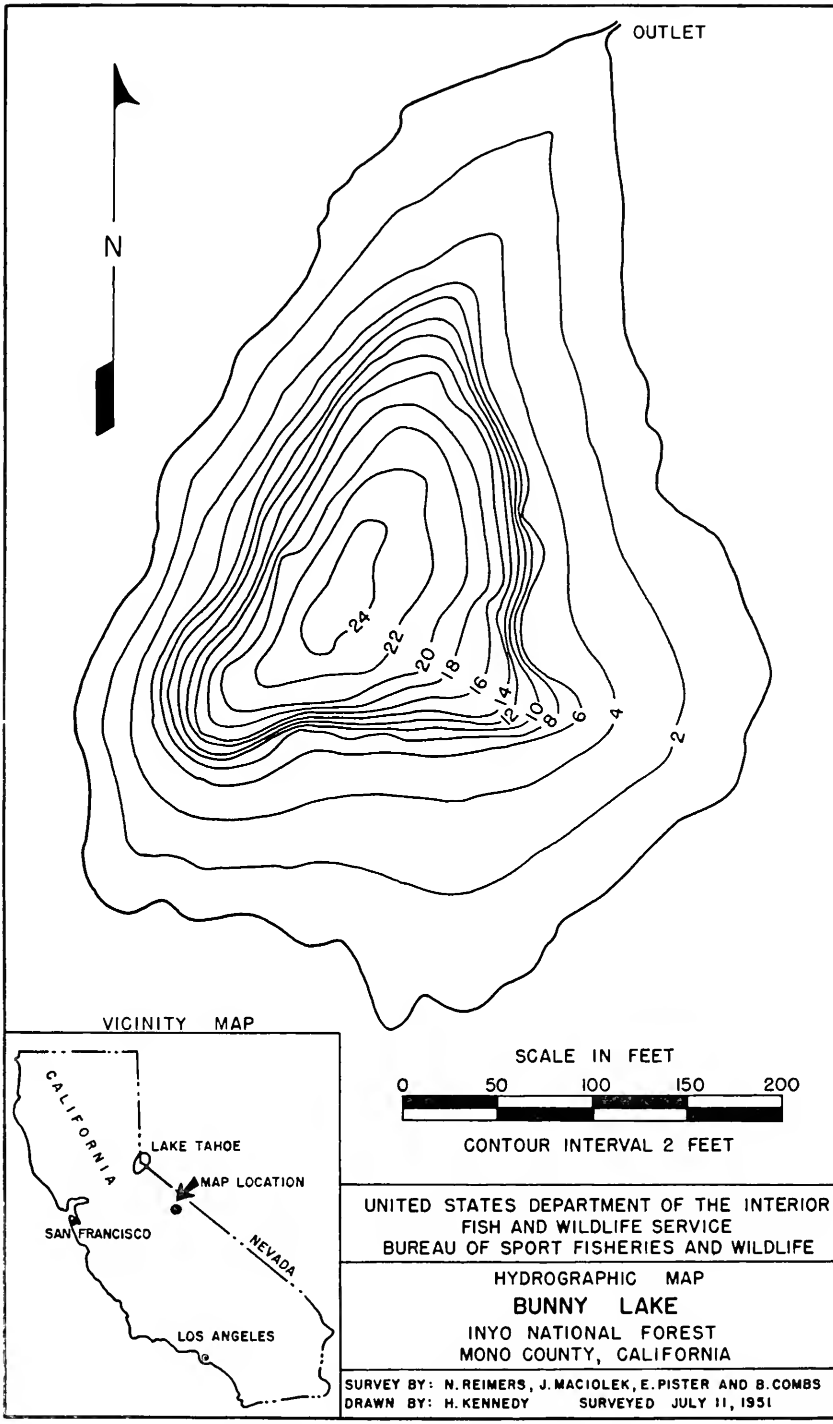


FIGURE 4. Hydrographic map of Bunny Lake.

influence of the shallows on solar warming of the water is suggested in the temperatures of July. The sharp change in temperature occurred just above six feet, and the six-foot contour is the line of radical change in depth (Figure 4). Two-thirds of the lake is less than six feet deep. The temperatures of August 29, 1957, almost uniform from bottom to surface at 44.2-44.6 degrees F., were the result of strong daytime wind mixing and the beginning of autumn cooling. At this time, nights were well below freezing and shore ice was present in the mornings.

## Chemical Features

In terms of dissolved substances, the water of Bunny Lake approaches chemical purity, due to its short time of contact with the poorly soluble substrate. Total dissolved solids, determined by evaporation of a water sample taken at middepth, amounted to 8.2 p.p.m. (parts per million) in 1951 and only 5.6 p.p.m. in 1953. Fractional analysis of constituents (Table 3) indicates the effect of a limited drainage from nearly bare silicate rocks (mainly quartz monzonite and granodiorite) on mineralization and nutrient quality of the water. Dissolved organic matter, represented by the weight loss on ignition of an evaporated sample of total solids, amounted to 0.2 p.p.m. or 3.6 percent of total solids. Determinations of pH varied between 5.8 and 6.3. Dissolved oxygen was present at 8.0 p.p.m. near the surface, 8.7

TABLE 3
Chemical Qualities of Mid-depth Water Samples From Bunny Lake
(Analyses Performed by Quality of Water Branch, Geological Survey, United States Department of the Interior)

| Item | Concentration in parts per million | |
|---|---|---|
| | 7/22/51 | 8/4/53 |
| Silica ($SiO_2$) | 2.9 | 2.1 |
| Iron (Fe) total | 0.02 | 0.07 |
| Calcium (Ca) | 0.7 | 0.2 |
| Magnesium (Mg) | 0.9 | 0.1 |
| Sodium (Na) | 0.6 | 0.5 |
| Potassium (K) | 0.3 | 0.1 |
| Nitrogen, ammonia ($NH_3$) | 0.028 | 0.064 |
| Nitrate ($NO_3$) | 0.2 | 0.1 |
| Nitrite ($NO_2$) | 0.2 | 0.0 |
| Boron (B) | <0.01 | * |
| Copper (Cu) | 0.10 | 0.00 |
| Manganese (Mn) total | <0.01 | 0.00 |
| Bicarbonate ($HCO_3$) | 6.0 | 4.0 |
| Sulfate ($SO_4$) | 1.3 | 0.1 |
| Chloride (Cl) | 0.2 | 0.1 |
| Fluoride (F) | 0.2 | 0.0 |
| Phosphate ($PO_4$) | 0.01 | 0.03 |
| Hardness as $CaCO_3$ | | |
| total | 6.0 | 0.9 |
| non-carbonate | 1.0 | 0.0 |
| Total dissolved solids | 8.2 | 5.6 |
| Loss on ignition | * | 0.2 |
| Specific conductance† | 8.9 | 8.0 |

* Not determined.
† Expressed in micromhos (reciprocal megohms) at 25 degrees C

p.p.m. at a depth of 22 feet, 8.4 to 8.6 p.p.m. at intermediate depths, and 9.7 p.p.m. near the main point of inflow at midday on July 31, 1951. The above concentrations are all greater than saturation values corrected for altitude, indicating the expected supersaturation of oxygen at all points in the lake.

## Aquatic Flora

A scanty phytoplankton, made up of diatoms, green algae, and blue-green algae, constitutes all of the nonbacterial aquatic plant life present in the lake. A significant part of this algal community exists in the form of periphytic aggregations on rocks and other submerged projections, and on the lake bottom. These forms were not sampled adequately with plankton nets, and no volume estimates of instantaneous phytoplankton density were made. Sampling of several depth strata with artificial substrates (Nielson, 1953) resulted in average 25-day accumulations of attached material (almost entirely algae) amounting to 2.98 milligrams (dry weight) per square decimeter of sampled surface. This figure is within the range obtained in similar samples of attached algae from other high lakes of the area during the same period, indicating that Bunny Lake does not have an exceptionally poor microflora as a result of its exceptionally low concentration of dissolved nutrients.

## Aquatic Fauna, 1951

### *Zooplankton*

Collections were limited to a few vertical hauls and surface tows with the plankton net, supplemented by hand netting at the surface. The collections were not used quantitatively, nor were they examined critically to determine all representative organisms; they were made only to establish the presence of macroplankters common to high lakes of the area, and to provide a basis for comparison of abundance before and after the introduction of trout. *Daphnia pulex*, a common cladoceran, and *Diaptomus signicauda*, a large red copepod, were the only prominent species found. A detailed examination would undoubtedly reveal an assortment of rotifers and protozoans, and possibly some smaller microcrustaceans. Both of the above species had been found in other lakes of the basin. Their density in Bunny Lake could not be estimated with any degree of accuracy by simple means. The cladocerans remained near the surface, easily avoided the plankton net, and were often driven by wind. In calm weather an estimated 10-30 individuals per cubic foot of surface water occurred over most of the lake, and after a strong wind there were up to 10 per cubic inch to a depth of several inches in areas of concentration at the leeward shore. The copepods apparently made irregular vertical migrations, and were often congregated just above the bottom. *Diaptomus* were roughly five times as abundant as *Daphnia* when all were near the surface.

### *Insects Observed From Shore*

In the months of July and August, mayfly nymphs (Ephemeroptera), caddisfly larvae (Trichoptera), and adult aquatic beetles (Coleoptera, Dytiscidae) were numerous in shoal areas. Examinations of 18 rocks

(total area 15.2 square feet) in water 2-20 inches deep produced 142 caddis larvae, 77 mayfly nymphs, and 24 beetles—an average of 16 visible insects per square foot. Hemipterous aquatics (water boatmen—Corixidae) and semiaquatics (water striders—Gerridae) could occasionally be found near shore. In mid-July many adult mayflies and newly emerged midges were seen over the lake, and a few winged terrestrial insects were usually afloat on the water.

### Bottom Organisms

Samples totaling 107 were taken with the Ekman dredge in 1951, 1952, 1953, and 1957. A brief analysis of the samples for each year is presented in Table 4. The lack of variety in the bottom fauna is typical of many alpine Sierra lakes in which brook trout live and sometimes thrive. Seemingly no lake is too poor to support a conspicuous population of midges (Diptera, Tendipedidae), the always dominant form whose larvae can be found in almost any kind of bottom material and without whose presence alpine trout stocking would surely fail.

TABLE 4

Percentage Abundance and Summer Standing Crops of Bottom Organisms in Bunny Lake, 1951-1957

| Year | Average percentage of total number constituted by | | | | Summer standing crop | |
|---|---|---|---|---|---|---|
| | Diptera (Tendipedidae) | Mollusca (Pisidium) | Annelida (Oligochaeta) | Other* | Average volume per sq. foot (cc.) | Average number per sq. foot |
| 1951 | 53.4 | 41.7 | 2.9 | 2.0 | 0.532 | 245 |
| 1952 | 57.4 | 39.7 | 2.3 | 0.6 | 0.404 | 184 |
| 1953 | 57.3 | 33.7 | 6.1 | 2.9 | 0.136 | 75 |
| 1957 | 53.3 | 30.0 | 16.7 | -- | 0.120 | 40 |

* Consisted of:
1951—Trichoptera, Ephemeroptera, Nematoda, Coleoptera, Hydracarina.
1952—Nematoda, Hydracarina.
1953—Nematoda.
1957—None.

*Pisidium*, a tiny, clam-like bivalve, is common in sandy bottoms but seldom is significant as trout food unless other foods are in very poor supply. Aquatic oligochaetes, which are angleworms on a small scale (most are less than one-half inch long in high lakes), are characteristically second or third in abundance but rarely appear in the trout diet because of their burrowing habit and inaccessibility.

In the 1951 samples from Bunny Lake, tendipedid larvae or pupae occurred in all 30 samples, *Pisidium* occurred in 57 percent of the samples, and oligochaete worms were found in 27 percent. Other organisms, listed in the footnote to Table 4, were relatively scarce and occurred occasionally in bottom samples. Although the average number of organisms per square foot (245) was slightly above the average for the 10 major lakes of Convict Creek Basin (217 per square foot; range 83-434), the volume per square foot (0.532 cc.) was far less than the basin

average (1.73 cc.), due to the small size of most midge larvae in Bunny Lake.

## INTRODUCTION OF TROUT

On August 25, 1951, the lake was stocked with an estimated 1,790 brook trout fingerlings which had been transferred from Mt. Whitney Hatchery (1950 spawn) and reared at Hot Creek Hatchery to 6.75 fish per ounce.[2] The trout averaged 6.6 centimers (2.6 inches) in total length and 4.2 grams in weight, as determined from a sample of 100 retained at the time of planting. They were transported in iced pack cans, without drugs, for about five hours over approximately 11 miles. All were released in good condition, and a post-planting observation gave no evidence of delayed mortality. A percolator dam of small and medium-sized stones was placed in the single outlet channel to prevent escapes.

## CHANGES IN THE ENVIRONMENT

In August, 1952, one year after the introduction of trout, all insects that formerly could be found near the shore were gone. It is probable that a radical reduction of these forms, which were nonburrowing and of relatively large size, took place in the two or three months before the first winter freeze-up. The remainder, if any, could have been consumed under the ice during the winter, or later in spring and early summer. Detailed searches of shallow-water areas, both exposed and under rocks, failed to turn up a single insect of the free-swimming or bottom-foraging type in 1952 or in later years.

Clear evidence of an inexorable reduction in the supply of other bottom organisms may be found in Table 4, a summary of data obtained from samples with the Ekman dredge. Although the various groups occurred in about the same proportions each year, the general abundance and volume of organisms declined—eventually to less than one-fourth of their original values. In connection with this decline, it is apparent from Table 4 that the small burrowing and interstitial organisms were not seriously threatened until the supply of mayflies, caddisflies, and beetles had been exhausted.

The larger plankters (*Daphnia* and *Diaptomus*) were very scarce in 1952, and were not found in later years except as occasional immature stages. It seems obvious that most were eaten as they became large enough to be recognizable as food items.

As shown in Table 3, some chemical differences in the water were indicated between 1951 and 1953. It is not known whether these represent yearly fluctuations due to weather, difficulties of analysis of such low concentrations, or alterations resulting in part from occupation by an overpopulation of fish. Possibly all three factors, as well as others, influenced the differences found. Concentrations of nearly all dissolved substances were lower in 1953 than in 1951.

[2] Both installations are eastern California trout hatcheries, operated by the California Department of Fish and Game.

## CONTENTS OF TROUT STOMACHS

The results of an examination of 40 trout stomachs (20 preserved in 1952 and 20 preserved in 1957) are given in Table 5. The food consisted almost entirely of immature midges, most of which were so small as to be barely visible to the human eye without an advantageous background. No stomachs were found empty in either year; all contained midges, and several contained nothing but midges. Mollusks (*Pisidium*) occurred in six of 20 stomachs in 1952, and in two of 20 in 1957. Other food items listed in the table appeared with still less frequency. The stomachs of other trout taken in the intervening years were examined grossly and were found to contain the same foods in similar proportions.

TABLE 5
Analysis of Stomach Contents of Brook Trout Taken One Year and Six Years After Stocking

| Item | August, 1952 | August, 1957 |
|---|---|---|
| Number of stomachs | 20 | 20 |
| Average volume* of stomach contents | 0.13 | 0.17 |
| Range in volume of stomach contents | 0.01-0.27 | 0.05-0.30 |
| Average numerical percentage† of all food organisms constituted by: | | |
| Aquatic Diptera | 95 | 98 |
| Mollusca | 5 | 1.5 |
| Aerial and terrestrial insects | Trace | 0.5 |
| Hydracarina | Trace | None |
| Other (Coleoptera larvae) | Trace | None |
| Number of stomachs containing sand and detritus | None | 15 |
| Average volume percentage† of stomach contents constituted by sand and detritus | - | 15 |
| Range in volume percentage of sand and detritus | - | 0 95 |

* Volumes expressed in cubic centimeters, determined by water displacement.
† From estimates and sample counts.

Although the proportions of different foods could not change appreciably in the five years between measurements, being strictly controlled by the dominance of midges and the relative scarcity or inaccessibility of the few other organisms, some changes in availability of food were indicated. Free-swimming water mites (Hydracarina) were found in trace amounts in most stomachs in 1952, but had disappeared from the diet in 1957. Mollusks were of minor importance as food in 1952 but occurred only rarely in the 1957 stomachs; evidently, only the visible individuals at the interface of water and bottom materials were cropped by the trout, for mollusks still made up nearly one-third of the bottom fauna in 1957. Midge larvae were less important than pupae in 1952, but much more important that pupae in 1957. Apparently the larvae were sought out in the bottom as they became fewer, since the bulk of detritus (mostly sand) accompanying the food in stomachs increased from nil in 1952 to significant amounts in 1957 (Table 5). The general decline in abundance, and likewise in availability, of all foods is reflected in the average volume of the stomach contents, which remained essentially the same despite the body growth and the reduction in numbers of trout.

## GROWTH OF TROUT

Trout were taken by angling each summer, except 1956, for body measurements and scale examinations. Changes in mean length and weight (Table 6) reflected the deterioration of the food supply, being near normal the first year and slight thereafter. Growth in length was only 9.7 cm. (3.8 inches) in six years, and over half this amount was added within a year after stocking. Body condition declined in accordance with the changes in body length, indicating a steady loss of weight in relation to length through the six years.

TABLE 6
Length, Weight, and Condition of Brook Trout in Bunny Lake

| Item | August 25, 1951 | August 12–14, 1952 | August 1–5, 1953 | August 12–29, 1954 | August 17–25, 1955 | August 26, 1957 |
|---|---|---|---|---|---|---|
| Number of fish | 100 | 76 | 33 | 46 | 27 | 37 |
| Mean length* | | | | | | |
| Centimeters | 6.6 | 12.0 | 13.3 | 14.9 | 15.2 | 16.3 |
| Inches | 2.6 | 4.7 | 5.2 | 5.9 | 6.0 | 6.4 |
| Range in length | | | | | | |
| Centimeters | 5.7–8.7 | 9.2–14.9 | 10.9–15.9 | 13.5–18.0 | 13.8–16.7 | 14.3–18.6 |
| Inches | 2.2–3.4 | 3.6– 5.9 | 4.3– 6.3 | 5.3– 7.1 | 5.4– 6.6 | 5.6– 7.3 |
| Annual length increase | | | | | | |
| Centimeters | . | 5.4 | 1.3 | 1.6 | 0.3 | 0.55 |
| Inches | -- | 2.1 | 0.5 | 0.7 | 0.1 | 0.2 |
| Mean weight (grams) | 4.2 | 15.4 | 20.5 | -- | 26.5 | -- |
| Mean condition factor (K)† | 1.099 | 0.870 | 0.851 | -- | 0.779 | -- |

* Total length; tip of snout to end of caudal rays.
† Based on total length.

In populations of trout characterized by normal growth patterns the scales may be used to advantage in the determination of age and rate of growth. Age in years is determined by counting the annual winter growth checks (annuli), which appear as crowded, broken, or overlapping rings (circuli) on the sculptured side of the scales. To ascertain the utility of scales from the stunted brook trout of Bunny Lake, microscopic examinations of the scales of known-age fish were made from the samples of all years except 1956. After two years of life the scales of most of these trout were useless, lacking all clues to the identification of third and later annuli. Others, which had the general appearance of normal structure but seldom could be interpreted even with resort to the knowledge of age, could not be relied upon to indicate the true age.[3]

[3] A similar situation was encountered in aging brook trout from other lakes in the basin. A few large-headed, thin trout, some of which were slightly above the age group III length range, were strongly suspected of being older. Their scales did not indicate greater age, but were disorganized in composition of circuli and were distinguishable from normal scales of age group III.

In contrast, the scales of a rainbow trout, inadvertently included in the lot and captured in 1957, developed a clearly recognizable annulus for each winter spent in the lake. Some of the best scales, which were carefully photographed but which nevertheless suffered somewhat as to reproduction of details, are shown in Figures 5 and 6.

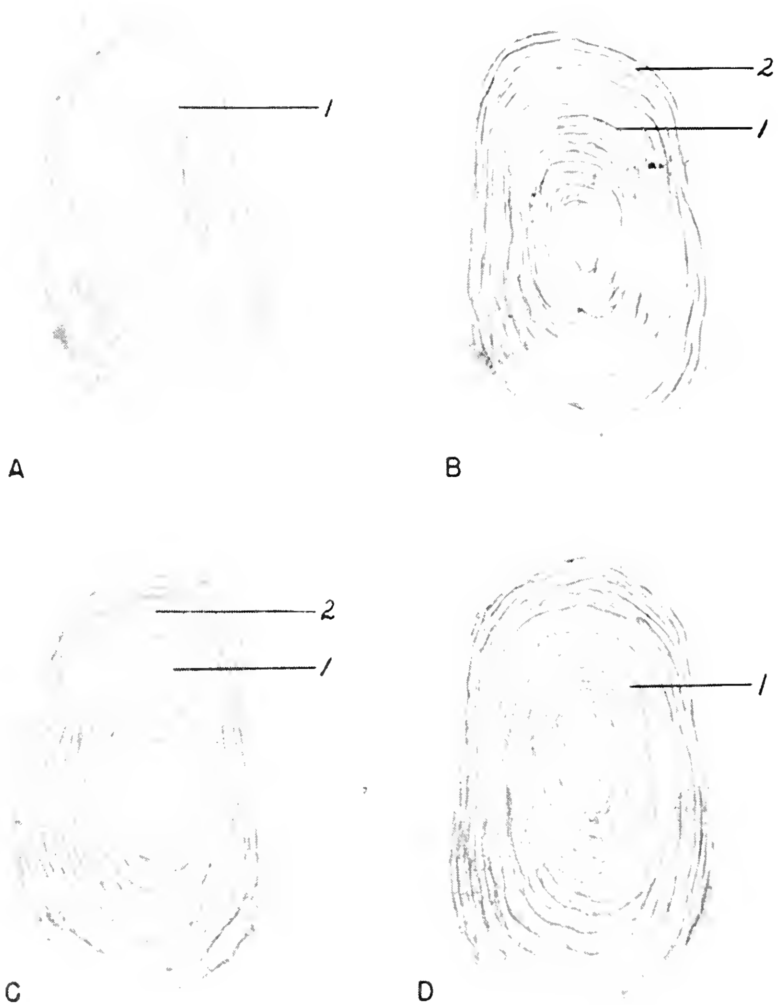


FIGURE 5. Scales of Bunny Lake brook trout of known age. Number of annuli visible should correspond with age group number; absence of pointer lines indicates that annuli are absent or cannot be located accurately. A, age group I; B, age group II; C, age group III; D, age group IV. *Photographs by E. P. Pister, December, 1957.*


FIGURE 6. Scales of Bunny Lake trout of known age. Absence of pointer lines indicates that annuli are absent or cannot be located accurately. A, scale of brook trout, age group VI; B, scale of rainbow trout, age group VI. *Photographs by E. P. Pister, December, 1957.*

## SEXUAL DEVELOPMENT OF TROUT

No trout eggs, young trout, or other evidences of natural spawning or attempted spawning were found in Bunny Lake. Examinations of sex organs indicated immaturity of all fish in 1952, maturity or near-maturity of several (both sexes) in 1954, and ripeness of a very few females in 1957. The distinction between maturity and immaturity, as made here, applies to the relative size and development of the gonads and their products. Females were considered ripe when most of their eggs were free and appeared ready for extrusion, and mature when the eggs approached ultimate size but were still in the membranes. The small size and number of "ripe" eggs, and the presence of shells from which the yolk had been resorbed, led to speculation that the state of nutrition and the physical condition of the trout were too poor for the production of fully developed, viable eggs. No ripe males were noted, although ripeness of males is common in August in other high lakes—presumably in more favorable living conditions. Most males were highly colored, and apparently were advancing in gonadal development in August of 1954 and later years.

## DISCUSSION

The foregoing account emphasizes the ease with which an over-population of trout, even one that is without reproduction and that is progressively reduced in size, can become impoverished in a limited environment. The extent of natural mortality is not known, but it is thought to have been moderate in view of the persistent hardship of the survivors. Sampling over the six years removed 319 trout, 17.8

percent of the original number. The food supply continued to diminish from year to year, while the numbers of trout visible and the effort required to catch them remained about the same. Bunny Lake was stocked at a density of just over 700 fingerling trout per acre, a figure that sometimes has been exceeded in routine replenishment stocking of lakes which already contained trout populations. In such a small, poorly productive lake, anything over 100 young trout per acre might well have been enough to stop the annual production of mayflies and other readily available food forms, as well as to crop the aquatic Diptera to the limit of the annual surplus.

In August, 1957, the trout were nearly seven years old, including hatching and rearing time. This is a longer life span than has been thought common for brook trout in the high alpine lakes, and it is certainly longer than what might be expected in the submarginal circumstances of Bunny Lake. Although it is conceivable that these trout gained extra years because of greatly reduced growth and a slower "rate of living," resulting from a minimum food supply, it seems more likely that such longevity is not in fact unusual but merely goes unobserved for one reason or another in lakes where it is attainable. In many lakes containing brook trout the angling pressure insures that very few trout will live longer than three or four years. In other lakes, predation by large trout adds to this early harvest. Wales and German (1956) reported that brook trout in Castle Lake, California, did not live beyond three years in the presence of large, predatory trout, but that a considerable number lived to seven years after the removal of the predators. In the less accessible back-country lakes occupied by brook trout only, more fish have an opportunity to grow older, though usually not much larger. Older fish are less likely to be recognized as such when they have grown but slightly and their scales have ceased to be legible indicators of age.

Bunny Lake is not a typical trout water, yet its exploitation by too many trout may be only a little less than typical of situations imposed upon some other alpine lakes in the effort to provide fishing from self-sustaining brook trout populations. Although the brook trout did not reproduce in Bunny Lake, they do spawn successfully in many other high lakes of the oligotrophic glacial type, in which their food is neither varied nor abundant. Without sufficient angling (the only form of predation upon trout in such lakes), reproduction in a closed system creates an overpopulation and results in a strain on the food supply. It does not take long, in a small lake, for an overpopulation to reduce its food supply to a level at which fish growth is very poor. Lakes filled with small trout do not attract heavy angling, and the metabolic adjustment of these trout is such that they may spend several years in half-starved condition unless drastic means are employed to remove them. It may then take years before a lake is capable of supporting anything but stunted fish regardless of what course—other than the removal of all, or nearly all, of the fish—is taken.

One conclusion from the work on Bunny Lake is that brook trout can get into food trouble rather easily in a small, lightly fished, rocky-basin lake and, once in trouble, can stay there for a long time on very

short rations. Another more speculative conclusion refers to the situation in which successful reproduction is a factor: the fishing has been good, but is tapering off; the catch is now made up of smaller fish, and young trout are seen; some laymen have decided to their own satisfaction that the lake needs restocking. The truth may be that the water is already crowded, in terms of its present food supply, and that the safest way to better the situation is to leave it alone or to remove some trout. Full recovery may be contingent upon the removal of all fish and the imposition of a resting period, followed by very light stocking.

## SUMMARY

Bunny Lake, a small, high-altitude, granite-basin type located in western Mono County, California, was surveyed and stocked with brook trout in 1951, then checked for changes in the environment and the trout in succeeding summers through 1957.

Within two years the trout had reduced the bottom-food organisms to less than one-third of original numbers; in six years, to less than one-sixth. Some food organisms, including the zooplankton, had virtually disappeared one year after the introduction of trout.

Growth of the trout was poor after the first year, negligible in the final two years. Growth the first year was 2.1 inches; in the remaining five years it amounted to 1.7 inches. Body condition declined radically in the first year, slowly thereafter.

Scales of these brook trout were not reliable indicators of age except in age groups I and II, but scales of a rainbow trout that had lived in the lake for six years displayed annuli commensurate with known age.

No reproduction of trout occurred. Sexual development appeared to have been delayed by poor nutrition and slow growth, and it is possible that full maturity, in terms of viable sex products, was not attained by the females at any time.

The results of the study seemed to indicate that some smaller alpine lakes, once depleted of food by overpopulation of trout, may be difficult to reclaim while trout remain in them.

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# RESPONSES OF BRUSH SEEDLINGS TO FERTILIZERS[1]

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## INTRODUCTION

Information on the response of brush plants to fertilizers is meager compared to that of agricultural crops, range grasses, or forest trees. Shrubs are a crop, and as such, may lend themselves to management on game ranges with techniques that are used for other crops. Fertilization is such a technique. This paper is an outgrowth of various experiments involving competition between brush and grass, the effects of prescribed burning on soil fertility, and the long-time influence of brush vegetation on soil properties and assays of wildland soils by means of fertility pot tests.

Data were supplied by four University of California research projects now in progress. One, in co-operation with the California Department of Fish and Game, is a segment of Federal Aid in Wildlife Restoration Project W51-R, entitled "Effects of Brush Removal on Game Ranges in California." The second is the Agricultural Experiment Station's contributing project to W-25, entitled "Ecology and Improvement of Brush Infested Range Lands." The California portion of this regional project deals with brush seedling establishment and growth in relation to soil fertility level. The third is an Agricultural Experiment Station project, "The Development and Handling of Browse Species for Domestic Livestock Use." These three projects are being conducted by the School of Forestry. Another project contributing to this paper is conducted by the Department of Soils and Plant Nutrition of the University of California. It is entitled "Determination of the Fertilizer Requirements of California Soils as Indicated by Pot and Field Studies with the Aid of Laboratory Studies."

## POT-CULTURE TECHNIQUE

The pot-culture technique was devised to measure the supply of available nutrients in soil (Jenny, *et al.*, 1950). Three nutrients—nitrogen (N), phosphorus (P), and potassium (K)—are added to pot cultures in several combinations. Indicator plants are grown for a standard period of time and relative yields are computed by comparing partial treatments—N + P, N + K, and P + K—with the full treatment containing all three elements. The relative yield represents the soil's ability to supply a given nutrient when other nutrients and soil

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moisture are not limiting. A high relative yield indicated by one partial treatment means the soil is well supplied with the nutrient not added.

Romaine lettuce, Atlas barley, and tomatoes have been used as standard indicator plants. They grow rapidly and seeds from homogeneous populations can be obtained commercially. Deficiency symptoms in these plants are well understood. However, barley is more sensitive to nitrogen than are lettuce or tomatoes, while tomatoes and lettuce are more sensitive to phosphorus than barley. Thus, different kinds of plants show different nutrient responses. Therefore, the relative yield concept can be used in another way: on the same soil, the relative yield represents either the requirement or the relative rate of uptake of a given nutrient by different plant species.

Except for the advantages of using domestic plants as indicators, the species naturally associated with the soil type may as well be used to test the soil nutrient status. Consequently, grasses can be used to test grassland soils (Gartner, *et al.*, 1957), pine seedlings to test forest soils (Vlamis, *et al.*, 1955), and brush seedlings to test brushland soils (Vlamis, *et al.*, 1958). While this procedure reduces the errors in calibrating pot tests with field responses, it may not differentiate between soils with small fertility differences, as is often required in research. The reason is that some grasses and most shrubs and trees are not as sensitive to nitrogen, phosphorus, and potassium as are the standard indicator plants.

## FERTILITY STATUS OF WILDLAND SOILS

Wildlands are the nonarable areas: forests, grasslands, and brush ranges. Most of the wildland soils are upland soils formed in place from various igneous and sedimentary rocks. They constitute profile groups VII, VIII, and IX among the classified California soils (Storie and Weir, 1952).

In a survey of about 100 upland soils, it was found that two-thirds were low in available nitrogen when lettuce was used as an indicator in pot tests. The no-nitrogen treatments yielded 30 percent, or less, of the full treatment on these soils. Also, two-thirds of the soils were low in available phosphorus. Here the no-phosphorus treatments yielded 20 percent, or less, of the full treatment. The 30 and 20 percent levels are not arbitrarily selected. Soils giving an $N_0$ lettuce yield of 30 percent or less and a $P_0$ yield of 20 percent or less would probably give field responses to these elements for agricultural crops. Nearly all of the soils investigated were adequately supplied with potassium.

When brush seedlings are used as the indicator plants, measurable differences also are found in the nitrogen and phosphorus supplying power of different wildland soils. Three brushland soils will serve as examples. In each case wedgeleaf ceanothus (*Ceanothus cuneatus*) seedlings were used for testing.

### Guenoc Loam

This soil was taken from eastern Tehama County, three miles southwest of Manton. The site was covered with a dense stand of wedgeleaf ceanothus. The Guenoc series (in profile group VII) is derived from basaltic flow rock and may support grass and woodland-grass as well as brush. The pot test with ceanothus showed this soil to be moderately

well supplied with nitrogen but very low in phosphorus (Figure 1). This soil has also been shown to be deficient in molybdenum and sulfur when tomatoes were grown under carefully controlled conditions (Vlamis, *et al.*, 1956).

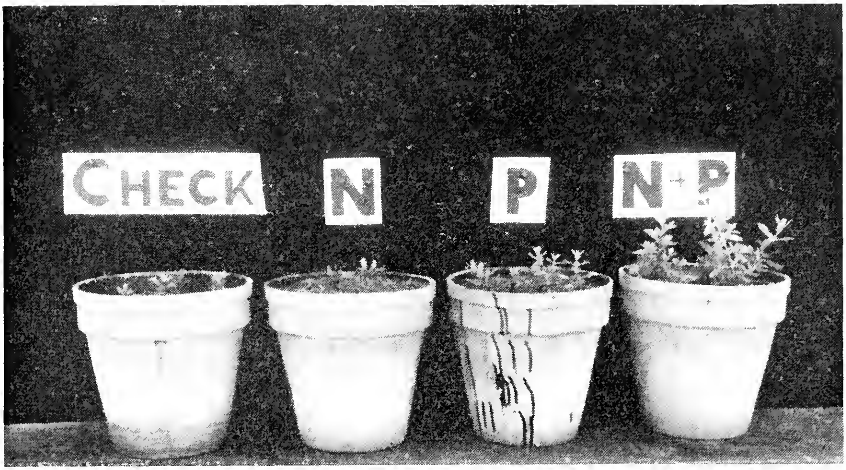



FIGURE 1. *Ceanothus cuneatus* seedlings growing on Guenoc loam. Pot test shows this soil to be moderately supplied with nitrogen and very low in phosphorus. Compare P treatment with N + P treatment to test nitrogen-supplying power of soil. Compare N with N + P to test phosphorus-supplying power.

## Phipps Clay Loam

This sample came from the vicinity of Quakenbush Mountain, two miles east of Clear Lake in Lake County. The site had a nearly pure, dense stand of chamise (*Adenostoma fasciculatum*). The Phipps series (profile group IX) is derived from weakly consolidated conglomerate, a soft sedimentary rock. The pot test showed this soil to be very high in available phosphorus and low in nitrogen (Figure 2).

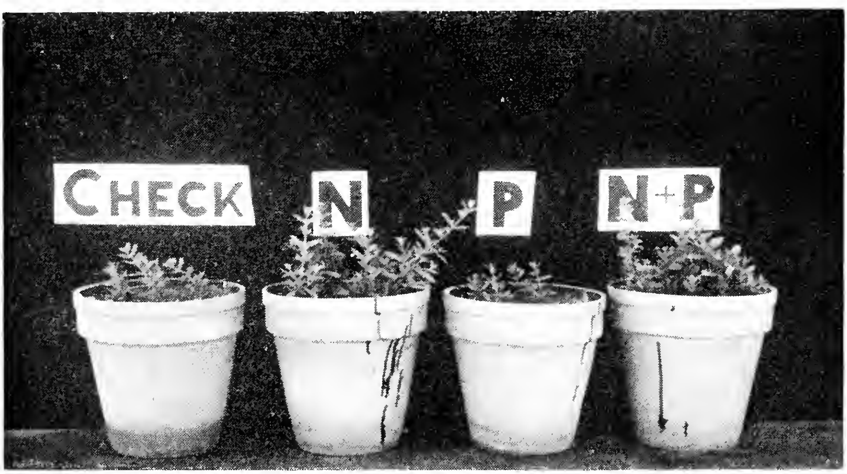



FIGURE 2. *Ceanothus cuneatus* seedlings growing on Phipps clay loam soil. Available phosphorus is high; available nitrogen is low.

## Maymen Gravelly Loam

The Maymen sample was taken from the northern boundary of the University of California Hopland Field Station in Mendocino County (Gowans, 1958). The site was covered with dense chamise. Soils of the Maymen series (in profile group VIII) are derived from sandstones or shales. They are nearly always brush covered. The pot test of this sample showed that both available nitrogen and phosphorus were very low (Figure 3).

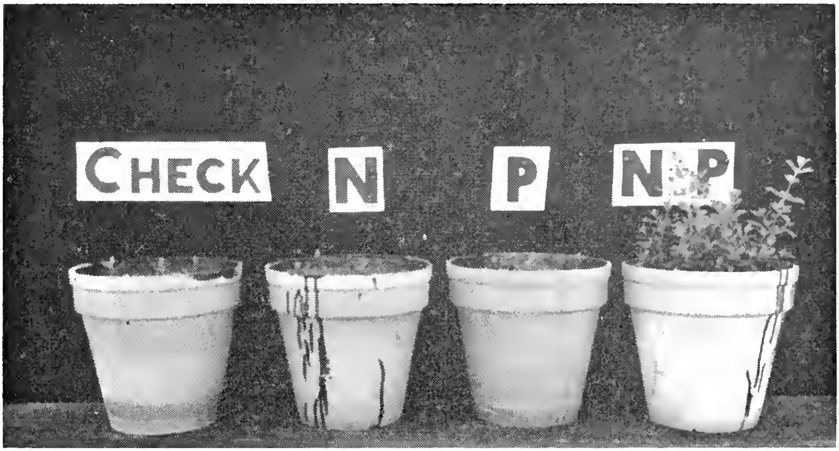



FIGURE 3. *Ceanothus cuneatus* seedlings growing on Maymen gravelly loam. Both available nitrogen and phosphorus are very low.

Relative yield for N and P on the three soils are given in Table 1, comparing the yields when brush seedlings, tomatoes, or grass are used as indicator plants.

TABLE 1
Relative Yields for N, P, and Check Treatment on Three Brushland Soils, Using Wedgeleaf Ceanothus, Tomato, and Annual Ryegrass as Indicator Plants *

| Fertilizer treatment | Soil type | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Guenoc | | | Phipps | | | Maymen | | |
| | Brush | Tomato | Grass | Brush | Tomato | Grass | Brush | Tomato | Grass |
| NP | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| P | 45 | 23 | 11 | 12 | 8 | 9 | 7 | 10 | 2 |
| N | 12 | 1 | 3 | 100 | 49 | 100 | 8 | 2 | 2 |
| Check | 12 | 0 | 3 | 20 | 8 | 8 | 6 | 3 | 1 |

* Expressed as percentage.

It is not to be inferred that the fertility levels which have been described are diagnostic of these three soil types or wildland soils in general. It is quite possible that as wide a range in fertility can be found within a soil classified as a single type as between series or types (Jenny, *et al.*, 1950).

## RESPONSE OF SEEDLINGS OF FOUR BRUSH SPECIES

In the foregoing section, relative yields were used to compare soils. The same species were grown on different soils. In this section relative yields are used to compare species. The same soil is the substrate for two or more species.

Seedlings of four shrub species have been studied in various experiments concerned with soil fertility problems. Two species of Ceanothus were used: wedgeleaf ceanothus and deerbrush (*Ceanothus integerrimus*). Two forms or varieties of deerbrush are involved: the Sierra variety (*C. integerrimus* var. *californicus*) and the Lake County form (McMinn, 1939). The other species are chamise and western mountain mahogany (*Cercocarpus betuloides*). All are important browse plants for deer and livestock in California.

About one week after emergence, seedlings were transplanted from natural populations in the field to flats. They were transported to Berkeley, and replanted in pots with different fertilizer treatments. There were five seedlings per pot. These were allowed to grow one or two years, depending upon the objective of the experiment. On the theory of Mitscherlich that yield per pot is not dependent on numbers of plants per pot, individual plants were not measured. After harvesting and drying, all five seedlings grown in the same pot were weighed together.

In each of the tests nitrogen was added to the pots at a rate equivalent to 200 pounds of N per acre, phosphorus at 300 pounds of $P_2O_5$, and potassium at 200 pounds of $K_2O$. The subscripts to the chemical symbols on the pot labels refer to these rates. The subscript "zero" means that element was not added to the pot. In one case, sulfur was an additional treatment.

Wedgeleaf ceanothus was grown on Holland sandy loam. This soil came from a ponderosa pine site near North Fork in Madera County. The Holland series is developed from acid igneous rocks under rainfall of 30 to 50 inches. The soils are extensive in area in the low altitude

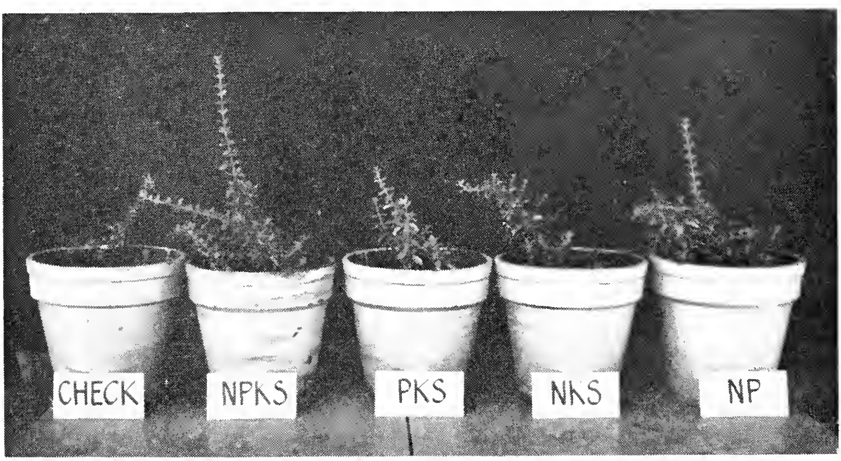



FIGURE 4. *Ceanothus cuneatus* seedlings growing on Holland sandy loam. This plant shows fair response to nitrogen (compare NPKS with PKS), some response to phosphorus (compare NPKS with NKS), and almost no response to potassium or sulfur (compare NPKS with NP).

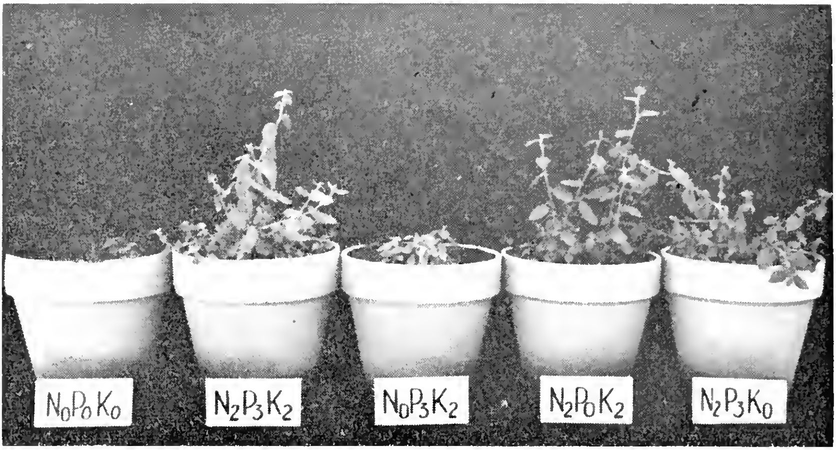



FIGURE 5. *Ceanothus integerrimus* var. *californicus* seedlings growing on Holland sandy loam. This plant shows good response only to nitrogen on this soil.

pine forests of the granite region. On this soil wedgeleaf ceanothus showed good response to N, some response to P, and no response to K (Figure 4).

The Sierra variety of deerbrush was also tested on the Holland soil. This variety grows naturally on the Holland soil type. After one year, seedlings responded only to nitrogen (Figure 5).

The Lake County form of deerbrush was tested on Salminas loam from Hobergs in Lake County. Salminas soils are formed from andesite, igneous rocks of volcanic origin. They are in a 30-60 inches rainfall belt and support good stands of ponderosa pine and other trees; also deerbrush and manzanita. The soil sample taken for the test was under ponderosa pine. The test showed good response to N, and some response to P and K (Figure 6).

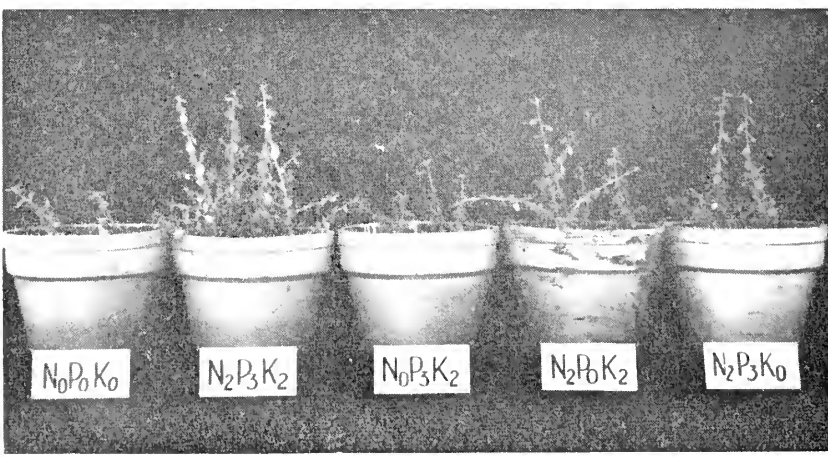



FIGURE 6. *Ceanothus integerrimus*, Lake County form, growing on Salminas loam. The test shows best response to nitrogen and least to potassium. Phosphorus seems to be readily available to this plant, in spite of phosphorus fixation in the soil

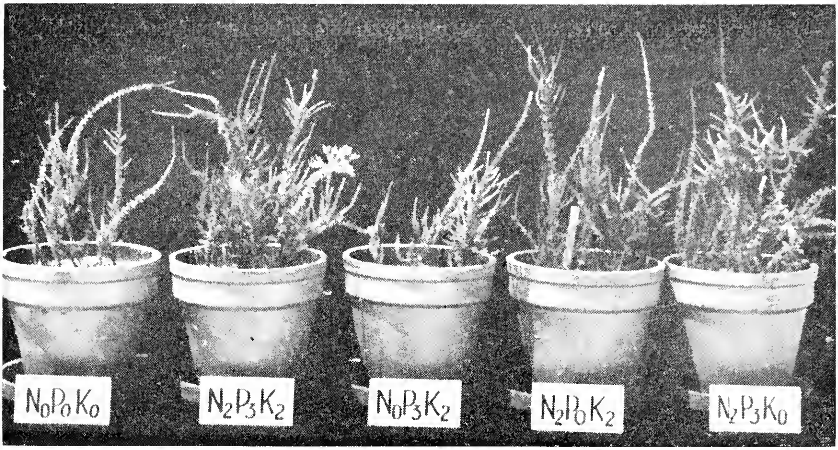



FIGURE 7. Two-year-old *Adenostema fasciculatum* seedlings growing on Maymen soil. There was some response to nitrogen but none to phosphorus or potassium.

Chamise was tested on Maymen soil. Chamise is the principal shrub species growing on the very extensive Maymen soil areas of the State. The soil sample was taken from Cow Mountain near Lakeport, Lake County. The pot test showed some response to N, none to P or K (Figure 7). These seedlings were allowed to grow for two years.

Western mountain mahogany was tested on the same Maymen soil. The seedlings also were grown for two years. Response to N was good; there was no response to P or K (Figure 8).

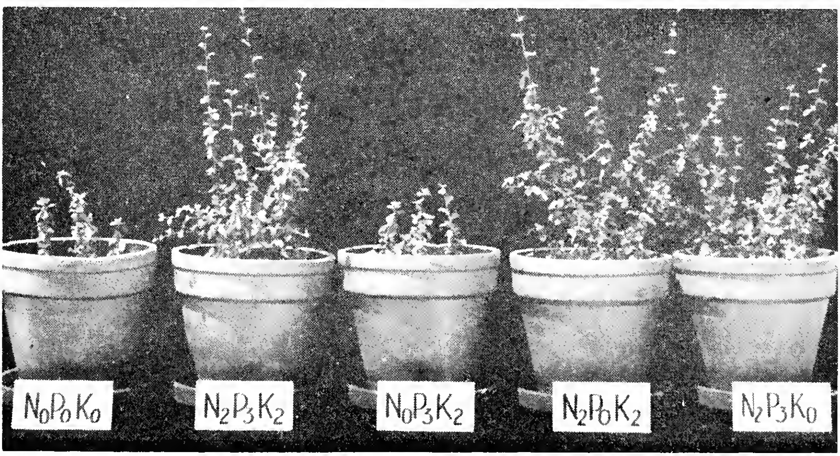



FIGURE 8. Two-year-old Cercocarpus *betuloides* seedlings growing on Moymen soil. This plant shows a very good response to nitrogen but no response to phosphorus or potassium.

Relative yields for these brush species are given in Table 2, along with barley and lettuce yields. Since the soils were not the same in each test, no direct comparisons can be made between all species; responses by the standard plants may be used to adjust those of the brush species.

TABLE 2
Relative Yields for Three Nutrients Expressed by Five Kinds of Brush. Barley and Lettuce Used on Each Soil for Comparison With Brush Seedlings.*

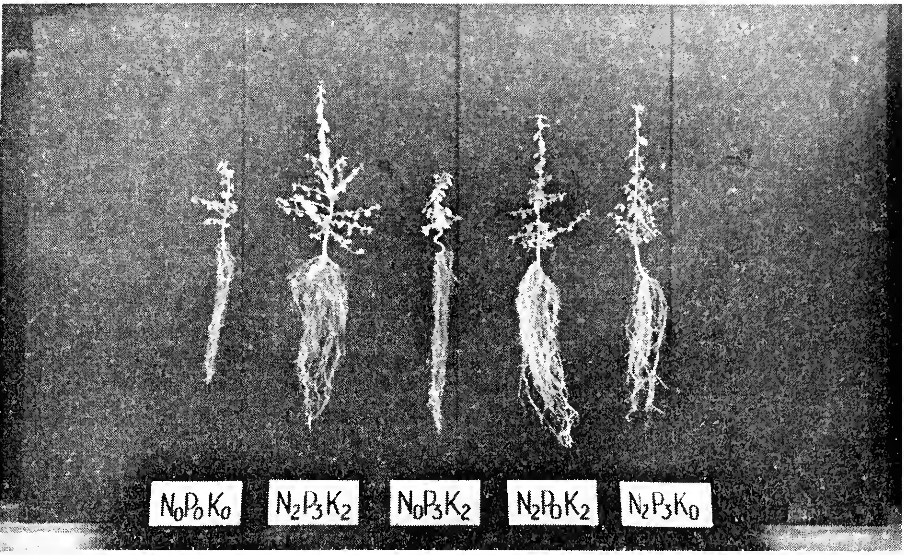
| Fertilizer treatment | Holland soil | | | |
|---|---|---|---|---|
| | Wedgeleaf ceanothus | Deerbrush Sierra var. | Lettuce | Barley |
| NPK | 100 | 100 | 100 | 100 |
| PK | 6 | 15 | 21 | 18 |
| NK | 51 | 93 | 7 | 22 |
| NP | 81 | 81 | 91 | 100 |
| Check | 7 | 12 | 4 | 14 |
| | Salminas soil | | | |
| | | Deerbrush Lake Co. form | Lettuce | Barley |
| NPK | | 100 | 100 | 100 |
| PK | | 19 | 12 | 21 |
| NK | | 47 | 12 | 21 |
| NP | | 86 | 85 | 95 |
| Check | | 18 | 12 | 17 |
| | Maymen soil | | | |
| | Chamise | Western mt. mahogany | Lettuce | Barley |
| NPK | 100 | 100 | 100 | 100 |
| PK | 29 | 17 | 27 | 21 |
| NK | 75 | 84 | 3 | 26 |
| NP | 89 | 89 | 87 | 89 |
| Check | 29 | 18 | 5 | 26 |

* Expressed in percent.

## RESPONSE OF BRUSH SEEDLINGS TO PRESCRIBED BURNING TREATMENTS

Some of the effects of prescribed burning on soil fertility have been determined for the Holland and Salminas soils. While burning does not add to the total nitrogen in the soil, it stimulates nitrification so that available nitrogen, as determined by pot tests, is higher (Klemmedson, *et al.*, 1958; Vlamis, *et al.*, 1955). Available phosphorus is also increased. These effects were first measured with lettuce and barley as indicator plants. Later it was determined that ponderosa pine and ceanothus seedlings showed similar results. Since these latter species are important for timber management and habitat improvement for game, their response to prescribed burning has immediate practical significance.

The prescribed burning technique results in two soil treatments. Broadcast burning has a low heat intensity because the fuel consists of the uppermost dry layer of needles, and coarser woody materials on the forest floor, distributed about as it fell. The fire burns rapidly over a given spot. The duff layer underneath is wet and will not burn; the



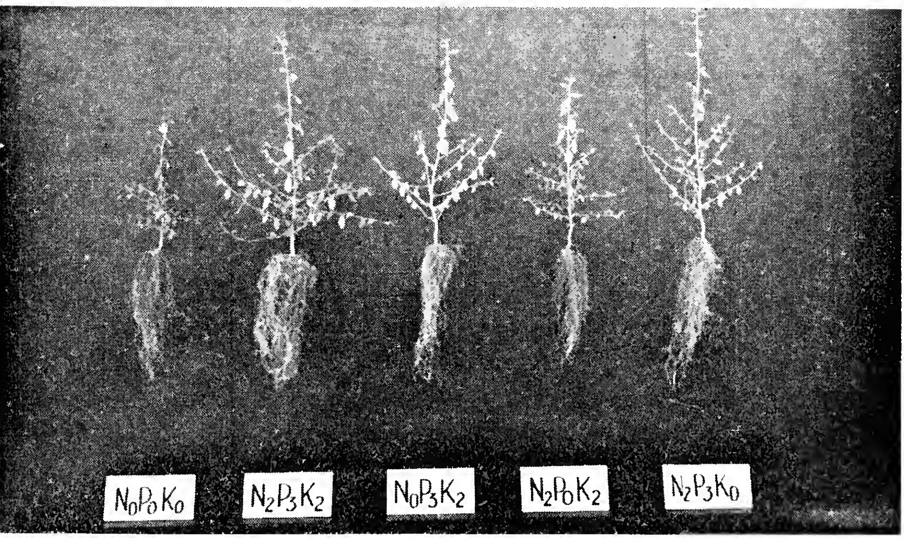




FIGURE 9. *Ceanothus integerrimus* seedlings, Lake County form, growing on unburned (upper) and burned (lower) Salminas soil. Seedlings on all treatments were larger on burned than unburned soil. Responses to nutrients on unburned soil were similar to those in Figure 6. There was no significant nitrogen response on burned soil but some response to phophorus.

soil surface just becomes warm. Cleanup burning consists of piling and burning brush and slash after broadcast burning. Fires burn intensely for several hours. Frequently, but not always, the duff layer burns to the mineral soil. The ashes represent a concentration of nutrients from a larger area. No more than five percent of the total area prescribed

burned receives this latter soil treatment. Pot tests using barley and lettuce as indicator plants show that the "fertility" effect of broadcast burning is intermediate between that of cleanup burning and no burning treatments (Vlamis, *et al.*, 1955). The Lake County form of deerbrush growing in pots of burned and unburned Salminas soil (Figure 9) shows the fertilizer effect on the intense burn. Soils broadcast burned only were not tested with brush seedlings. Prescribed burning increased the relative yield of no-nitrogen treatment from 20 to 70 percent (Table 3). Absolute response was equivalent to an N application of nearly 200 pounds per acre. However, it should be remembered that in practice this high rate is "applied" to no more than five percent of the area.

TABLE 3
Relative Yields of Two Brush Species on Burned and Unburned Soils

| Fertilizer treatment | Wedgeleaf ceanothus | | Deerbrush Lake Co. form | |
|---|---|---|---|---|
| | Holland soil | | Salminas soil | |
| | Unburned | Burned | Unburned | Burned |
| NPK | 100 | 100 | 100 | 100 |
| PK | 6 | 36 | 19 | 71 |
| NK | 21 | 10 | 47 | 66 |
| NP | 81 | 98 | 86 | 86 |
| Check | 7 | 22 | 18 | 53 |

Increase in phosphorus available to deerbrush as a result of prescribed burning is not marked. This is because the Salminas soil fixes much of the phosphorus which would otherwise become available from the ash. Plants are not able to take up this fixed phosphorus. The Sierra

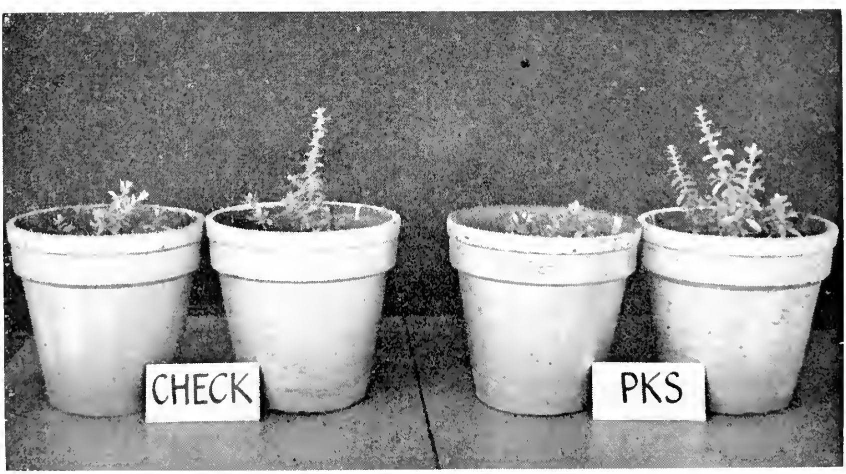



FIGURE 10. *Ceanothus cuneatus* seedlings on Holland soil in response to burning. For each of the two no-nitrogen treatments, check and PKS, the unburned soil is on the left. Burning increases the available soil nitrogen supply.

variety of deerbrush growing on burned Holland soil (not illustrated) did show an increase in available phosphorus over the unburned condition. The Holland is not a phosphorus fixing soil.

Wedgeleaf ceanothus also reflects the increase in available nitrogen from burned (Figure 10, second and fourth pots from left) compared to unburned (first and third pots) Holland soil.

## ROOT NODULES AND NITROGEN FIXATION

During the first season deerbrush seedlings respond to added nitrogen with vigorous growth. In pot tests, the plants with $N_0$ treatments grew slowly. In one experiment deerbrush seedlings were not harvested at the end of the first season. It was noted that during the second year one plant out of five in a pot with $N_0P_0K_0$ and two in a pot with $N_0P_3K_2$ outgrew the others by a wide margin. The latter pot was selected, the roots of each plant removed intact from the soil and separated carefully. Large nodules were noted on the two large plants, one small nodule on a small plant, and no nodules on the other two small plants (Figure 11).

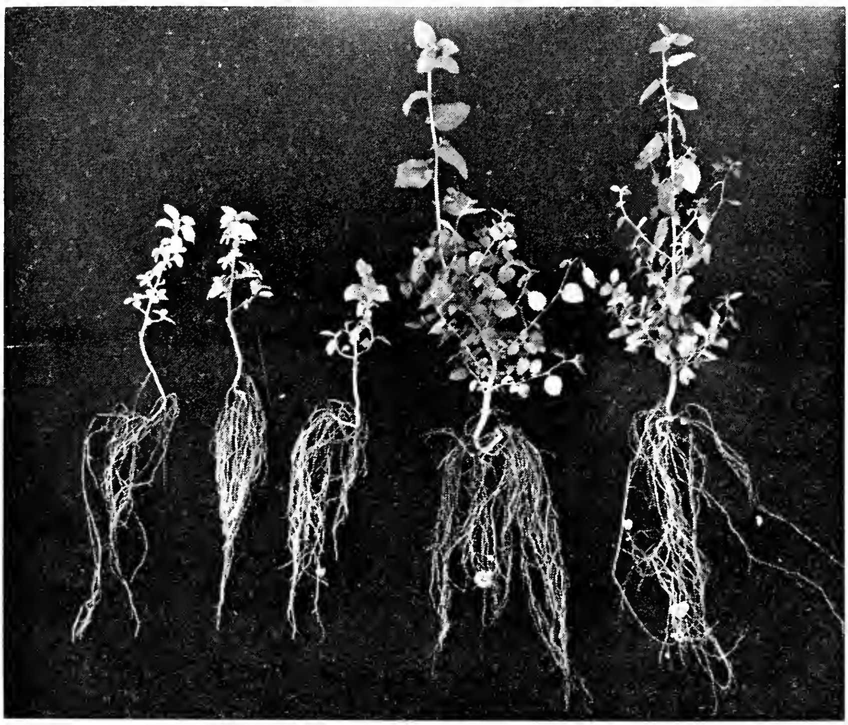


FIGURE 11. Five two-year-old *Ceanothus integerrimus* seedlings from the same pot: no-nitrogen treatment on Holland soil. Nitrogen was being supplied to the two large plants by nitrogen fixing organisms in the nodules on the roots. Nodules were beginning to form on the roots of the small plants; one is clearly visible on the center plant.

Dry weights were taken of the shoots of all the plants in a similar experiment. These can be summarized as follows (Vlamis, *et al.*, 1958):

| *Treatment* | *Average weight per plant (gms.)* | *Range in weight (gms.)* |
|---|---|---|
| (1) No nitrogen added, no nodules | 0.6 | 0.4- 0.7 |
| (2) No nitrogen added, nodules present | 5.3 | 5.0- 5.7 |
| (3) Nitrogen added, no nodules | 9.9 | 8.2-12.0 |

Inoculation was accidental so not all the plants had nodules. Presumably, the second group of plants took up nitrogen fixed by symbiotic organisms in the nodules. These nodules were not active during the early growth stages of the seedlings. The third group, however, had nitrogen from the start. As with legumes, nodule formation and symbiotic nitrogen fixation are inhibited by high nitrogen concentration.

Many, if not all, species of the genus Ceanothus have nodules. It is possible that these shrubs are able to fix significant amounts of nitrogen when growing on low fertility soils. The influence of phosphorus fertilization on nodulation by ceanothi has not been studied. These remarks also apply to leguminous shrubs including chaparral pea (*Pickeringia montana*), redbud (*Cercis occidentalis*), Scotch broom (*Cytisus scoparius*), bush lupine (*Lupinus albifrons*), and deerweed (*Lotus scoparius*), and possibly also to alders (*Alnus* spp.).

## ROLE OF FERTILIZERS IN GAME RANGE MANAGEMENT

The biological aspects presented in this paper should be interesting to an imaginative game manager. A few of the possibilities can be enumerated.

Like other plants, brush seedlings respond to fertilizers. This simple phenomenon can be used to advantage for brush establishment on infertile soils or on ranges where shrubs have difficulty in growing. However, we know little about nutrient responses by older plants. Generally, the older the plant the smaller or slower the responses to a fertilizer treatment. There is no reason why this principle would not apply to brush.

The fact that shrubs respond somewhat differently to some nutrients, e.g. phosphorus, than do grasses or other herbaceous plants gives us a tool with which to manipulate two major kinds of vegetation. It may be possible to control the density of shrubs by competition, using fertilizers high in phosphorus and low in nitrogen. The ratio of response to phosphorus, grass to brush, is about 4 to 1. Time of application is a selective agent, too.

Composition of the brush stand may be altered. We should not fix upon P and N alone. Gypsum, lime and other amendments or nutrients may be equally important in favoring one species over another. It would seem that where ceanothi or palatable legumes are present and desired, nitrogen should be withheld.

The palatability of certain species can be improved by fertilization. Phosphorus and potassium raise the sugar content of plants; nitrogen tends to prolong the period of succulence. Depending on differential uptake of nutrients by shrub species, preferences may be changed. The overall effect would be an increase in palatability of total forage or a

reduction in selectivity by browsing animals. This has great possibilities as a management tool if it affects deer movements and extension of home range.

Diets on winter ranges may be manipulated in accordance with new concepts about food, nutrition, and stressor agents. Production of browse and herbaceous forage can be directed toward carbohydrates or proteins by manipulating the phosphorus or nitrogen supply, respectively, of the plants.

The merits of prescribed burning should be re-evaluated from the standpoints of being a cheap method of fertilizing, for quick establishment of seedlings, its influence on palatability of such species as manzanita, and improvement of browse quality (Lay, 1957). This is in addition to values in fire hazard reduction, inducement of crown sprouting, fire treatment of seeds, and cover conversion (Biswell and Schultz, 1956; 1958).

Benefits accruing not directly to game, especially watershed values and erosion control, can be enhanced with fertilizers (Hellmers, *et al.*, 1955). Fertilizers may well benefit more than one kind of crop in the multiple use management of wild lands, but they may also be detrimental to a single use, such as game, if their effects lead to overuse by livestock or higher rates of water use by vegetation and, consequently, result in less drinking water for wildlife.

Most of these ideas are hypothetical and need further study. Naturally, besides the biological aspects of brush fertilization, consideration must be given to the social implications—economics, policy, and game management ethics—but these are outside the scope of this paper.

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# NOTES

## SOUTHERLY OCCURRENCES OF THREE NORTHERN SPECIES OF FISH DURING 1957, A WARMWATER YEAR ON THE CALIFORNIA COAST

During the latter part of 1957, three fishes, common in northern ocean latitudes, were taken to the southward of their usual reported ranges. These catches are especially interesting in view of the fact that water temperatures along the California Coast in 1957 were several degrees higher than the average of the last several years.

On September 11, 1957, a Pacific halibut, *Hippoglossus stenolepis,* 44 inches in total length, was caught in an otter trawl operated from the fishing vessel *Friendship* (Capt. Kenneth Dall), in 480 feet of water, four miles south of Pt. Piedras Blancas (latitude 35° 35′ N.). The halibut appeared in a rockfish catch consisting mainly of bocaccio and chilipepper. The only previous record of Pacific halibut, southward of Monterey Bay, is that noted by Walford (1928), who reported a 14-pound specimen caught in 600 feet of water on November 18, 1927, on the southeast side of Santa Rosa Island, Southern California.

On October 2, 1957, two specimens of the grunt-sculpin, *Rhamphocottus richardsoni*, 68 and 70 millimeters total length, were taken in a modified otter trawl operated from the City of Los Angeles boat *Prowler*, while conducting dragging operations as part of a surveillance program of the Hyperion Outfall, off the town of Santa Monica, Southern California. A 10-minute drag in 252 to 324 feet of water, with a 1½-inch mesh net, resulted in a catch of the two grunt-sculpins and 427 other fish, representing, all together, 25 species in 12 families (Table 1).

Grunt-sculpins are common intertidally in the Pacific Northwest, but according to Mr. W. I. Follett, California Academy of Sciences, references to their occurrence in California are either rare or vague. The two or three authentic records for years prior to 1957 were from Monterey Bay and vicinity. However, during January, 1957, an angler left a live grunt-sculpin with the "Sea Wonders Alive Aquarium" in Crescent City. It had been caught on hook and line at a depth of about 90 feet in the ocean near there. This live specimen was identified at the aquarium by biologist Ed Best, on February 1, 1957.

Thus, the Santa Monica Bay occurrences (latitude 33° 56′ N., longitude 118° 33′ W.) extend the southerly range for this species by about 250 miles. These two specimens, collected by biologist John L. Baxter, have been deposited in the fish collection of the University of California, Los Angeles.

A Pacific cod, *Gadus macrocephalus*, 20 inches in total length, was caught December 2, 1957, in an otter trawl operated from the fishing

vessel *Meldon* (Capt. Neil Burton) at a depth of 660 feet, six miles south of Pt. Piedras Blancas. This fish also appeared in a rockfish catch consisting mainly of bocaccio and chilipepper. The capture of this specimen extends the southerly range of Pacific cod some 50 miles, from latitude 36° 18′ N., in the vicinity of Pt. Sur (Phillips, 1953) to latitude 35° 33′ N., in the vicinity of Pt. Piedras Blancas.

TABLE 1
List of 429 Fishes Taken During 10-minute Drag October 2, 1957, in Santa Monica Bay at 252 to 324 Feet

| Family | Scientific name | Number taken | Standard lengths (range in millimeters) |
|---|---|---|---|
| Chimaeridae (chimaeras) | *Hydrolagus colliei* | 7 | |
| Bothidae (lefteyed flounders) | *Citharichthys sordidus* | 92 | 70–164 |
| Pleuronectidae (righteyed flounders) | *Microstomus pacificus* | 12 | 87–202 |
| | *Lyopsetta exilis* | 1 | 136 |
| | *Pleuronichthys decurrens* | 1 | 189 |
| Embiotocidae (viviparous-perches) | *Zalembius rosaceus* | 75 | 70–126 |
| | *Cymatogaster aggregata* | 4 | 100–107 |
| | *Rhacochilus vacca* | 1 | 108 |
| Scorpaenidae (rockfishes) | *Sebastodes semicinctus* | 121 | 86–147 |
| | *Sebastodes dalli* | 61 | 77–100 |
| | *Sebastodes chlorostictus* | 3 | 136–140 |
| | *Sebastodes jordani* | 2 | 120–140 |
| | *Sebastodes goodei* | 1 | 260 |
| | *Sebastodes rubrivinctus* | 1 | 77 |
| Zaniolepidae (combfishes) | *Zaniolepis frenata* | 9 | 132–171 |
| | *Zaniolepis latipinnis* | 1 | 157 |
| Cottidae (sculpins) | *Icelinus tenuis* | 17 | 42–85 |
| | *Icelinus quadriseriatus* | 2 | 47–51 |
| | *Icelinus fimbriatus* | 1 | 83 |
| | *Icelinus filamentosus* | 1 | 208 |
| Rhamphocottidae (grunt-sculpins) | *Rhamphocottus richardsoni* | 2 | 55–58 |
| Agonidae (poachers) | *Xeneretmus triacanthus* | 6 | 141–156 |
| Batrachoididae (toadfishes) | *Porichthys notatus* | 3 | 56–129 |
| Bathymasteridae (ronquils) | *Rathbunella hypoplectus* | 1 | 132 |
| Ophidiidae (cusk-eels) | *Otophidium taylori* | 1 | 237 |

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—*J. B. Phillips, Marine Resources Operations, California Department of Fish and Game, May, 1958.*

## INTESTINAL FLUKES AS A POSSIBLE CAUSE OF MORTALITY IN WILD TROUT

From time to time the Department of Fish and Game receives reports from its own employees and from interested sportsmen that large numbers of dead trout have been found in certain lakes. Malicious poisoning, "winterkill," or dynamiting are frequently suggested as causes

of such mass mortalities. Two such cases of mass mortality have been investigated by the writer, and he believes both were due entirely or in part to an intestinal fluke, *Crepidostomum*.

In Lower Gumboot Lake, Siskiyou County, many dead rainbow trout (*Salmo gairdnerii*) were found soon after the ice had melted in the spring of 1956. Some of these fish were examined, and their intestines were found to be seriously inflamed. In the contents of the intestines were large numbers of flukes. Again, in the spring of 1957 many rainbow were found dead in this lake, and their intestines were similarly parasitized. These fish were six to nine inches in length and may have been "catchable" trout planted during the previous year. In late October, 1957, dead trout, both rainbow and eastern brook trout (*Salvelinus fontinalis*), were found on the bottom of Castle Lake, Siskiyou County. These fish also ranged in size from about six to nine inches. Most of these dead fish were rainbow. In both Lower Gumboot Lake and Castle Lake the rainbow were of the domesticated, fall-spawning strain.

One freshly dead rainbow was recovered from Castle Lake, and in its intestine were found 446 flukes. A gill net was set in this lake, and 17 eastern brook and 4 rainbow were caught. Examination of these showed 10 of the eastern brook and all of the rainbow contained several to many flukes. Although most of the fish examined were parasitized, they did not contain excessive numbers of worms, and it is assumed that they were not being seriously injured by them. In trout with very large numbers of flukes the intestines are inflamed, and it is possible that the flukes are the direct or indirect cause of death. The numbers of such fish killed in these epizootics can only be guessed. However, it is probable that the losses would have an appreciable effect upon the fishing.

The freshly dead rainbow from Castle Lake also had large numbers of the protozoan, *Hexamita*, in the intestines. The effect of these protozoa is unknown, but experience with hatchery trout of these sizes would indicate that the *Hexamita* were probably not the primary cause of death.

The worms from the Castle Lake fish were definitely identified as belonging to the genus *Crepidostomum*, and probably to the species *farionis*. Necessary comparative material was not available for positive identification to species. Several of these flukes, preserved in formalin and in a contracted state, ranged from 0.87 to 1.19 mm. in length, and from 0.42 to 0.70 mm. in breadth. When alive, they may be three times that long.

Crawford (1943) states that when the eggs of this fluke are expelled from the fish, the miracidia emerge from the egg and enter the fingernail clam, *Pisidium*. Later the cercaria emerge from the clam and enter the mayfly nymph, *Ephemera*. The trout eat the nymphs and so become parasitized. The writer does not know what genera of mayflies are present in Castle Lake.

It is recommended that in cases of unusually heavy mortalities of trout in lakes, the intestinal contents of freshly dead fish be examined. The adult worms are just large enough to be visible without a magni-

fying glass. If placed in water, they can be seen moving slowly about. The walls of the intestine of heavily parasitized trout will be highly inflamed.

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—*J. H. Wales, Inland Fisheries Branch, California Department of Fish and Game, March, 1958.*

# REVIEWS

*Seals, Sea Lions, and Walruses. A Review of the Pinnipedia*

By Victor B. Scheffer; Stanford Univ. Press, Stanford, Calif., 1958; x + 179 pp., 15 figs., and 32 plates. $5.

The author writes from a store of first-hand experience as he has specialized in marine mammals during much of his association as a biologist with the U. S. Fish and Wildlife Service. Mr. Scheffer has written many scientific and popular articles primarily dealing with these interesting pinnipeds or fin-footed animals.

This is the first major systematic account of the pinnipedia since they were reviewed by Allen in 1880 and catalogued by Trouessart in 1897-1905. During the past 50 years man's knowledge of life in the sea and his understanding of animal evolution has increased greatly. For this reason the author felt there was a need to re-examine the pattern of distribution and variations displayed by the seals and seal-like animals of the world.

The author has clearly defined the characteristics of the pinnipeds as well as the manner in which they have adapted themselves to an aquatic environment. He has traced the evolution of the pinnipeds down through the ages from their pre-carnivorous ancestors and has placed their possible ancestral home in the northern hemisphere, where today, one finds two of the three families and 12 of the 20 genera. The synoptic key presented in the text will enable the reader to identify the different genera by their most distinctive features.

The bookshelf of any serious mammalogist or biologist would be incomplete without this excellent book. Although not written for popular consumption, it will make excellent reading for anyone interested in marine mammals. The extensive list of references would in itself be a welcome addition to biologist's library.—*Emil J. Smith, Jr., California Department of Fish and Game.*

*Snakes in Fact and Fiction*

By James A. Oliver; the MacMillan Company, New York, N. Y., 1958; 199 pp., 20 black and white photographs. $4.95.

In the animal kingdom, snakes provide one of the most fascinating subjects for discussion. Dr. Oliver, Curator of Reptiles of the New York Zoological Society, is well acquainted with the subject.

The book is flavored with personal narratives of the author and other experienced naturalists and herpetologists.

Dr. Oliver dispels many superstitions and myths concerning serpents. From Iormungander, the terrible serpent of Norse mythology to Kipling's story of "Rikki-tikki-tavi" he discusses the origin of popular beliefs about snakes and then presents actual observations and information collected from many sources.

Those who have wondered about the famed Hopi Indian's Snake Dance, the snake charmers of India and why there are no snakes in Ireland will find some interesting reading also.

The information presented should ease the mind of many who have heard and believed erroneous and exaggerated stories of snakes. Dr. Oliver states that in the United States "more people die each year as a result of slipping in the bathtub or being struck by lightning than die from snake bite."

Of interest to the herpetologist are facts concerning: the largest snakes; prodigious meals taken; the most aggressive and dangerous; reproductive habits; family life and denning observations.

The highly touted snake killers are exposed. These include the mongoose, King snake and roadrunner. The author points out that man has persecuted snakes beyond all reason and is their greatest enemy.

The black and white photographs are excellent although sparing. Additional photos of other snakes mentioned in the text would have enhanced the format.

Many of the questions frequently asked about snakes are answered in this book written in an interesting and readable style. The reviewer recommends this book to all. *Trevenen I. Wright, California Department of Fish and Game.*

### *San Francisco Bay: A Pictorial Maritime History*

By John Haskell Kemble; Cornell Maritime Press, Cambridge, Maryland, 1957; xv + 195 pp., illus. $10.

This volume will find a permanent place in the maritime history of San Francisco Bay. In 18 chapters the author presents a truly outstanding assemblage of photographs, drawings, and paintings with which he has reconstructed maritime San Francisco from its discovery to the great bustling ports which presently surround the bay.

The various phases of maritime activity are treated in separate chapters; each is carried through in chronological order. A sample of chapter headings might include. "The River Ports," "Building and Repairing Ships," "Whaling and Deep Sea Fishing," "Americans in the Cape Horn Trade" and "The Navy in the Bay."

The second chapter, "The Port of San Francisco" is especially interesting. Here, with actual photographs, one is shown the almost unbelievable transformation which the tiny hamlet on Yerba Buena Cove underwent in a few short years following the discovery of gold in California. On pages 12 and 13 the author presents a panoramic view of the waterfront taken in 1851 with the ships creating a veritable forest of masts. In striking contrast, pages 28-31 present the Port of San Francisco and the Embarcadero as we know it today.

The old timer who knew the waterfront and various ports around the bay between 1900 and 1920 can reminisce to his heart's content while turning the pages of this volume, for there are stirring pictures throughout; the sailing fleet wintering in the Oakland Estuary; British grain ships anchored in Richardson and Suisun Bays; the steamboats, Chrysopolis, Reform, Fort Sutter, Pride of the River, Newark (later the Sacramento), Eureka, the largest passenger ferry in the world, and many others; the whaling fleet; the shipyards of years gone by; and the now absent Italian lateen rigged fishing boats or "feluccas." Though these phases of maritime activity are now history, they are vividly brought to mind and by the slightest imagination the reader can picture the bay as it was many years ago.

A full chapter is devoted to the deepwater steamships. The captions describe the development of the intercoastal and transoceanic steamship business, while the photographs illustrate advancements in steamship building.

A few pages are devoted to sport on the bay, and although most forms are represented, sport fishing is conspicuously absent. The party boat fleet in particular has been a colorful part of the bay maritime life for many years. More recently, the bay has literally been dotted by many forms of small private fishing boats from skiffs to yachts; yet this activity has been overlooked. It is interesting to note, however, on page 77, incidental to a photograph of the steamer Antelope tied alongside the Oakland Long Wharf, the tremendous number of anglers fishing for smelt (about 1870), a scene now quite unlikely to be duplicated.

Generally speaking, the emphasis throughout the book, has been on the changes in the maritime activities around the bay, but the author also accentuates vessel nomenclature. Thus the reader encounters such descriptive terminology as brig, brigantine, bark, barkentine, sloop, yawl, schooner, scow, frigate, etc. Since these terms and several others relating to ship rigging are used, it is unfortunate that a paragraph or two was not included giving the definition of these terms. The interested reader would do well to look them up in a good dictionary as they are encountered to get the fullest enjoyment of this book.

The book is a must for those interested in the maritime history of the bay. The choice of photographs, drawings and paintings is excellent and each is adequately captioned. The identification of vessels is particularly notable.

The great martime commerce displayed throughout the book recalls the envisioning of Captain John Fremont when he first named the Golden Gate.—*John E. Skinner, California Department of Fish and Game.*

### *Surf Fishing*

By Vlad Evanoff, The Ronald Press Co., New York, 1958, v + 120 pp., illus. $2.95.

Surf fishing novices can learn much about the sport by reading this book, and even the experienced fisherman will pick up some valuable tips. The reader will find descriptions of conventional and spinning tackle, natural baits and artificial lures, instructions on how to cast and how to fish different types of beaches, as well as the author's techniques for catching some of the popular surf species. Good line drawings illustrate baits, lures, other tackle, items, and many of the fishes taken by surf anglers.

Descriptions of fishing methods, baits, and surf fishes primarily refer to the Atlantic coast although there is a limited amount of specific information on Pacific Coast fishing. However, California surf fishermen might be pleasantly surprised with their results if they were to try some of the east coast gear and techniques. This reviewer has observed eastern "transplantees" enjoying fine success with the "fish finder rig" described on page 48, although such gear is not commonly used by California veteran anglers.

The barred surfperch, Southern California's most popular surf species, fails to receive mention. Other popular California surf species excluded from specific attention are the redtail and calico surfperches, the cabezon, and the opaleye.

This book will provide enlightening reading for all who are interested in surf fishing and it provides complete "how to do it" instructions for the Atlantic coast surf fisherman.—*Norman Abramson, California Department of Fish and Game.*

### *Elements of Mathematical Biology*

By Alfred J. Lotka; Dover Publications, Inc., New York, N. Y., 1956; 465 pp., 36 tables, and 72 figures. $2.45.

This book is one of the excellent reprints from classic works in science prepared by the Dover Press. The book is paper bound but the quality of the paper and binding are equal to cloth bound books.

*Elements of Mathematical Biology* was first published in 1924 as *Elements of Physical Biology*. At that time many of the concepts of Lotka must have seemed new, at any event, no one before had synthesized the knowledge of physical explanations for living things.

Lotka analyzed what was known in such fields of investigation as growth, interspecies equilibrium, the water cycle, phosphorous cycle, carbon dioxide cycle and evolution. Other subjects seem properly addressed to physicists and mathematicians and geologists. Some subjects covered are now common property of limnologists, zoologists and botanists. The book is a summary of many basic ideas in science which have been accepted for many years, but this is a single place where these ideas have been brought together to help explain life processes.

Notes for the book were first collected in 1902 and the topics were further developed in 1907. In explanation Lotka is mechanistic, the common approach today. He does not believe we should name things until we discover them and once again we follow this line of reasoning. But 56 years ago some of these ideas must have been young.

It would be an assistance to the reader to be familiar with symbols used in the art of mathematics. In addition the reader should have some knowledge of several sciences. Lotka wrote, in what seems today, an old fashion manner, with classical references and quotations in several languages. In all it is certainly worth the reader's time to read those chapters which deal with his own field.—*Robert R. Bell, California Department of Fish and Game.*

### *Portage Bay*

By Paul I. Wellman; Doubleday & Co., Inc., Garden City, New York, 1957; xi plus 240 pp. illus. $4.00.

This book is not written specifically for the biologist. It is not technical. It is written for the fishermen who long for the call of the loon, big water, unpredictable weather, and the fish of the setting—the walleyed pike. There are other species of fish, such as the musky, smallmouth bass, northern pike, and lake trout, which enter into this fisherman's tale.

Four anglers annually travel 13,000 miles to spend two weeks together fishing on Lake of the Woods near the Northwest Angle, the most northern part of the United States. One is an Oregon rancher. Two are Kentuckians—one a race horse breeder, the other a sporting goods man. The fourth is the writer, a Los Angeles resident.

The setting is at Portage Bay camp, a summer guest camp, located on the north shores of Lake of the Woods on a one-half mile portage to Shoal Lake. At this camp, between the guests and the host (as well as among the guests themselves, particularly the above four) occurs a round of practical jokes, tall tales, fishing camaraderie, and convivial drinking.

Although written primarily as a fisherman's diary, there is a seasoning of local history which makes interesting reading. The story is told of Father Jean Pierre Aulneau, a Jesuit priest, who was murdered on the lake by local Indians more than two centuries ago. It tells of his founding Fort St. Charles in 1732, its later abandonment, and the return of the fort to the forest. The bones of Father Aulneau and the remnants of fort fireplaces were discovered by Jesuit confreres in 1908.

There is a lesson in poetry given the four fishermen by a local Indian guide having "an expression of unspeakable stupidity." Much to the amazement of the four fishermen, Green Feather (the guide) points out some of the mistakes made by the poet Longfellow in his Song of Hiawatha. The poem does not scan, i.e., Longfellow took poetic license more through ignorance than by purpose in setting the meter in trochaic dimeter, an impossibility with the Ojibwa tongue. Ojibwa words are most often accented on the antepenult, whereas the accent in the poem is on the penult. The name of the hero, Hiawatha, is not Ojibwa; however, the legend is pure Ojibwa. The name of the Ojibwa hero was Manabozho. Hiawatha was a Mohawk (Iroquois) chief, one of the founders of the dread Iroquois Confederation.

Those interested in angling success will be particularly impressed with the results of a few hours of fishing. The four men caught 311 fish represented by 20 northern pike, 9 bass, 3 perch, 1 musky that got away, and the remainder of which were walleyes. Only four fish of the entire catch were kept, indicating these men as true fishermen at heart. It is not often that one may read an account of freshwater angling so successful as to exhaust its participants. After five hours of fishing at a time when the fish were still striking vigorously, the men had to quit. This is the account of one day. As in all fishing, there were days of abysmal luck—*Robert L. Butler, California Department of Fish and Game.*

### *Living Silver*

By Burns Singer; Houghton Mifflin Co., Boston, Mass., 1958; 232 pp. illus., $3.75.

Mr. Singer is a biologist turned poet and in writing this book he has used many colorful phrases to describe the sea, the fishing vessels, and the many fishes hauled from the sea. He studied zoology at Glasgow University and was subsequently employed by the Scottish Home Department's marine laboratory at Aberdeen. Working there he gained considerable knowledge of the methods used to convert the "living silver" of the sea into the "pound sterling."

The general description of the main fishing methods—trawling, Danish seine, ring-nets, drift-nets, and long line—of the British fishing industry is generously sprinkled with scientific facts about the species sought by each type of gear. Also included is a slight mention of the economics of the marketing of the catch after landing. By far the most space is devoted to trawling. Beginning with the Scottish school for training tyro trawlermen and working up to a typical trawling trip. The description of the British side-set trawls will be somewhat more familiar to Atlantic Coast trawlermen than to the Pacific Coast fishermen who prefer to set their trawls over the stern of the vessel.

The line drawings show little resemblance to actual fish, although this may be due to the illustrator trying to follow the written descriptions, such as, the catfish "looks rather like a punch-drunk boxer who has lost a couple of front teeth." The book should appeal to anyone interested in the sea and particularly to those making a living from the sea—*E. A. Best, California Department of Fish and Game.*

***Treatise on Marine Ecology and Paleoecology. Volume 1: Ecology***

Edited by Joel W. Hedgpeth; the Geological Society of America, New York, 1957; Memoir 67, viii + 1, 296 pp. and frontispiece, profusely illus. with text figures, plates, and maps. $12.50.

One has to see and read this volume to believe it could happen. It could easiest be described with a lengthy string of Hollywoodian superlatives, but in a way, even this would not do justice to such a monumental work.

The 29 chapters on assorted subjects in the field of marine ecology plus the 36 sections of annotated bibliographies comprise over 1200 pages of text by some 60 authorities. The sequence of chapters is natural and easy to follow. Terminology is nowhere complex, and the text is relatively easy to read and comprehend. Although an adequate index permits finding specific items with a maximum of speed and efficiency, casual browsing can be a rewarding experience.

A classification of marine environments is presented in an early chapter on the subject. Grouped nearby are chapters on the concepts of ecology and the obtaining of ecological data. These are followed by discussions on the various physical, chemical, and ethereal factors affecting or capable of affecting marine ecology: solar radiation, lunar periodicity, salinity, temperature, oxygen, carbon dioxide, nutrient elements, etc.

A lot of "meat," food for thought, and very fine reference data appear in subsequent chapters discussing major habitat types and the ecological niches contained therein. These chapters, on bottom communities, rocky intertidal surfaces, sandy beaches, coral reefs, submarine canyons, deep sea and abyssal depths, and estuaries and lagoons, if consulted prior to initiating a study will do much to start one on the right track and keep him there once started.

Special sections have been included on the Baltic Sea, Black Sea, Caspian Sea, Aral Sea, and Sea of Azov. Contributions on fluctuations in littoral populations and mass mortalities in the sea contain important data and more than 100 pages (of fine print) are devoted to annotated bibliographies of all of the major marine groups and phyla.

All of those associated with the publication of this invaluable addition to the field of marine ecology are to be commended for their efforts. A copy of this volume should adorn the "within-easy-reach" shelves of every person's library who is actively engaged in working with marine organisms—*John E. Fitch, California Department of Fish and Game.*

***Fishes of the World***

By Edouard Le Danois (Translated from the French) The Countryman Press, Woodstock, Vermont; 190 pp., 80 photogravure plates, 30 color photographs. $12.50.

This book is full of fine, large photographs of fishes. The text rambles through the fishes of the world, grouped by northern, temperate, tropical, and southern regions; with a final brief section on deep-sea forms. Sidelights of commercial fisheries and life histories, woven through the discussion, make for easy reading.

The translation is adequate, for the most part, although it occasionally falls short. North American ichthyologists will be intrigued by some of Le Danois' concepts of our fauna. He says of Pacific salmon, "Now they pair and the females hollow out furrows with strokes of the tail and lay their eggs in them. The males stay close to the spawn, immediately fertilize it and cover it with fine gravel. At intervals of several minutes for two whole months the pair ceaselessly renew their efforts."

The French printer who made the 30 dramatic color plates in this book merits particular praise—*Alex Calhoun, Department of Fish and Game.*

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## A



## B



## C



## D

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## STATE OF CALIFORNIA
### FISH AND GAME COMMISSION

Notice is hereby given that the Fish and Game Commission shall meet on January 2, 1959, in the California State Building, First and Broadway, Los Angeles, California, to receive recommendations from its own officers and employees, from the department and other public agencies, from organizations of private citizens, and from any interested person as to what, if any, regulations should be made relating to fish, amphibia, and reptiles, or any species or subspecies thereof, in accordance with Section 208 of the Fish and Game Code.

FISH AND GAME COMMISSION

WM. J. HARP
Assistant to the Commission